Investigation of Role of Rab GTPases in Fruit Ripening in Tomato (*Solanum lycopersicum* L.)

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Abstract

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops cultivated worldwide. Rab GTPases are involved in the processes of intracellular membrane traffic, protein secretion and signal responses for targeting of molecules for secretion into the cell wall. Silencing these genes is likely to affect fruit softening by blocking the export of a range of cell wall modifying factors. A cDNA clone from the tomato fruit encoding a protein homologous to *rab11/YPT3* was developmentally regulated during fruit ripening. Antisense fruit changed colour but failed to soften normally (Lu *et al*., 2001). The aim of the project is to investigate the role of Rabs and other elements of intracellular membrane trafficking in secretion of cell wall modifying factors associated with fruit softening.

Sixty genes encoding Rab GTPases in tomato were identified by *in silico* analysis by screening the EST databases and were classified as Rab GTPases according to Release 12 of TIGR Tomato Gene Index. The genes were grouped into 8 different functional classes ranging from Rab1, 2, 5, 6, 7, 8, 11 and Rab18. A phyletic tree was constructed which indicated all the genes were falling into 8 different clades belonging to each class. A comprehensive *in silico* analysis of other genes involved in intracellular membrane trafficking like Rab GEF, GAP, GDI, and other genes like ARF, ARL, RAN, ROP, SAR (Vernoud *et al*., 2003) was done by using the same databases. The results showed that there are thirteen ARF GTPases, eighteen ARL GTPases, seven SAR GTPases, eight ROP GTPases, and a total of nine RAN GTPases in tomato. The Rab GTPase interacting partners like GAP, GDI and GEF were also identified. The results revealed that there are twenty three Rab GAPs, two Rab GEFs and a total of six Rab GDIs were found.

The information about the genes was updated throughout the course of the present investigation right from the TIGR DFCI TGI release11,12 and 13, SGN-Unigene Build 1 and 2 as well the latest release of tomato whole genome sequence database at SGN from SGN ITAG-1 to ITAG-2.4. After the final analysis, a total of 55 Rab GTPases were grouped into eight different sub classes with five Rab1/D, five Rab2/B, four Rab5/F, three Rab6/H, four Rab7/G, five

Rab8/E, twenty five Rab11/A and four Rab18/C. Solyc Rab11/A was further classified into 6 sub groups from A1 to A6. The results of classification led to identification of seven RabA1, six RabA2, one RabA3, five RabA4, five RabA5 and one RabA6. The phylogenetic analysis of the tomato Rab GTPases and a comparative analysis with Arabidopsis Rab GTPase confirmed the groups and classes. Similarly four RAN, ten ROP, twelve ARF, four SAR, six ARL GTPases were identified. In case of ARF GTPases seven ARFA, four ARFB, and one ARF C was found. The regulators of small GTPases identified in tomato had one RAB GEF, two ROP GEF, four RABGDI and five ROP GDI, twenty two RabGAP, 2 RAN GAP, nine ROP GAP and nineteen ARF GAP. Comparative phyletic analysis of all the above with *Arabidopsis* genes confirmed the classification.

The molecular and phenotypic characterization confirmed the presence of antisense *LeRab11a* transgene and the phenotypes were heritable. The textural analysis to characterise the pattern of fruit softening of the wild type and transgenic plants by compression and penetration test from different fruit developmental stages was done and the results showed a sequential pattern of delayed softening and the transgenic fruits were firmer than wild type at the later stages of fruit ripening and had enhanced shelf life and reduced susceptibility to fungal diseases.

The relative expression profiles generated by qPCR also showed the pattern of differential expression of Rab11 group of GTPases in wild type and transgenic fruits. Along with this the latest freely available expression database were also used to generated the expression profiles of Rab GTPases during different developmental stages not only in wild type Ailsa Craig (AC++) but also in Heinz cultivar as well as in *Solanum pimpinellifolium*.

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Abbreviations

µg	micro gram
AC++	Ailsa Craig
ACC	1-aminocyclopropane-1-carboxylic acid
ACO	ACC oxidase
ADP	Adenosine diphosphatase
Ara	*Arabidopsis thaliana* Rab genes
ARF	ADP ribosylation factor
ARF-bp	ARF binding
ARL	ARF like
ATPase	Adenosine triphosphatase
AtRab	*Arabidopsis thaliana* Rab genes
B	Breaker
B+ (N)	Breaker + (N) number of days
BLAST	Basic Local Alignment Tool
CaMV 35S	Cauliflower mosaic virus 35 S subunit
CCV	Clathrin coated vesicles
cDNA	Complementry DNA
CDS	Coding Sequence
CHS	Chalcone synthase
cnr	Colourless non ripening
COPI, II	Coat protein complex-I and II
C-terminal	Carboxy terminal of protein
dATP	2'deoxyadenosine 5'-triphosphate
dCTP	2'deoxycytidine 5'-triphosphate
DEPC	diethylpyrocarbonate
dGTP	2'deoxyguanidine 5'-triphosphate
DNA	Deoxyribonucleiotide
dNTPs	2' deoxynucleotide 5'-triphosphate
dUTP	2'deoxyuridine 5'-triphosphate
E. coli	*Escherichia coli*
EDTA	Ethylenediamine tetra acetic acid
EE	Early Endosomes
ER	Endoplasmic reticulum
Exo	Exocyst elements
g	grams
GA	gibbrelic acid
GAP	GTPase Activcating protein
GAPDH	Glyceraldehydes 3-phosphate dehydrogenase

GDF	GDI displacement Factor
GDI	GDP dissociation inhibitor
GDP	Guanosine diphosphate
GEF	Guanine nucleotide exchange factor
GTP	Guanosine triphosphate
GTPase	Guanosine triphosphatase
GGTase	Geranylgeranyl transferase
IAA	Indole-3-acetic acid
IMG	Immature green
kb	Kilo base
LE	Late endosome
Le Rab	*Lycopersicum esculentum Rab* gene
M	Molar
MG	Mature green
MW	Molecular weight
mRNA	Messenger RNA
ng	nano gram
nor	Non ripening
NPTII	neomycin phosphotransferase II
N-terminal	amino terminal of protein
ORF	open reading frame
PCR	polymerase chain reaction
PE	pectinesterase
PG	polygalacturonase
PM	plasma membrane
PME	pectin methylesterase
Poly(A)	polyadenylate
pra	*Pisum sativum* Rab gene
PTGS	Post transcriptional gene silencing
qRT-PCR	quantitative real time polymerase chain reaction
Rab	Ras like in Rat Brain
Rab GGTase	Rab geranylgeranyl transferase
Ran GTPase	Ras like nucleolar GTP binding protein
RE	Recycling endosome
REP	Rab escort protein
RER	Rough Endoplasmic Reticulum
RHO GTPase	Ras homologue Rac- like / GTPase
rin	Ripening inhibitor
RNA	Ribonucleic acid
RNAi	RNA intereference

RNase	Ribonuclease
ROP	RHO like GTPase of Plants
Rpm	Revolution per minute
RR	Red ripe
RT-PCR	Reverse transcription polymerase chain reaction
SAM	S-adenosyl methionine
SDS	Sodium dodecyl sulphate
Sl Rab GTPase	*Solanum lycopersicum* Rab GTPase
SNAP	Soluble NSF attachment protein
SNARE	Soluble N-ethylmaleimide sensitive factor attachment protein receptor
TAE	tris/acetate/ETDA buffer
TGN	Trans golgi network
Ti	Tumor inducing
Tris	Tris(hydroxymethyl) amino methane
t-SNARE	Target- SNARE
UV	ultra violet
VAMP	Vesicle associated membrane protein
v-SNARE	Vesicle-SNARE
WT	Wild type
ypt3	Yeast *S. pombe* transport 3 gene
YFP	Yellow fluorescent protein

1. Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops cultivated worldwide. It is the second most commonly grown vegetable crop in the world after potato. The botanical classification of tomato has had an interesting history; it was placed in the genus *Solanum* by Linnaeus in 1753 as *Solanum lycopersicum* L. The name is derived from 'lyco' meaning wolf, 'persicum' means peach, i.e., "wolf-peach" and *esculentum* means edible. However, in 1768 Philip Miller placed it in its own genus, and he named it *Lycopersicon esculentum*. This name was in breach of the plant naming rules. The current botanical classification is as under

Kingdom:	Plantae
Subkingdom:	Tracheobionta
Division:	Magnoliophyta
Class:	Magnoliopsida
Subclass:	Asteridae
Order:	Solanales
Family:	Solanaceae
Genus:	Solanum
Species	*S. lycopersicum*
Chromosome:	2n=2x=24
Genome Size:	950Mb

1.1 Economic importance and nutritional value

Tomato is an ideal research material for the physiological, cellular and biochemical studies. It is easy to cultivate, with a short life cycle. It can be cultured *in vitro* and is responsive to tissue culture enabling plant regeneration for genetic transformation (Hille *et al*., 1989; Giovannoni, 2001, 2004; Seymour *et al*., 2008; Mueller, 2009; Gerszberg *et al*., 2015). Several mutants are available which play an important role in physiological studies (Gray *et al.*, 1994; Klee and Giovannoni, 2011). Per capita fresh market tomato consumption continues to increase in much of the world and tomato products are found in a great variety of processed foods. Tomato fruit is a rich source of vitamin A and C and contains the natural antioxidant lycopene, it has also been found beneficial in preventing cancer (Boehm *et al.*, 2016; Marti *et al*., 2016). The nutritional values of tomato are given in table 1.1.

Table 1.1 Nutritional value of tomato.

Nutrient Proximates	Value per 100 g	Vitamins	Value per 100 g	Minerals	Value per 100 g
Water	94.52 g	Vitamin C	13.7 mg	Calcium (Ca)	10 mg
Energy	18 kcal	Thiamin	0.037 mg	Iron (Fe)	0.27 mg
Protein	0.88 g	Riboflavin	0.019 mg	Magnesium(Mg)	11 mg
Total lipid (fat)	0.2 g	Niacin	0.594 mg	Phosphorus (P)	24 mg
Carbohydrate	3.89 g	Vitamin B-6	0.08 mg	Potassium (K)	237 mg
Total dietary Fiber	1.2 g	Folate	15 µg	Sodium (Na)	5 mg
Sugars, total	2.63 g	Vitamin A (RAE)	42 µg	Zinc (Zn)	0.17 mg
Lycopene	2573 µg	Vitamin A (IU)	833 IU	Copper(Cu)	0.059 mg
beta Carotene	449 µg	Vitamin E (alpha-tocopherol)	0.54 mg	Manganese(Mn)	0.114 mg
Lutein + zeaxanthin	123 µg	Vitamin K (phylloquinone)	7.9 µg		

Basic Report 11529 (USDA), Tomatoes, red, ripe, raw, year round average. National Nutrient Database for Standard Reference Release 28 slightly revised May, 2016.

According to FAOSTAT (2013 for 2011 and 2016 for 2013)[1], the top producers of tomatoes in world were China, India, US, Turkey and Egypt as shown in table 1.2.

Table 1.2: Top Tomato Producers in world (2011 & 2013)

Top Tomato Producers (Production in million tonnes)		
Country	**2011**	**2013**
China	48.57	50.6
India	16.82	18.3
United States	12.52	12.6
Turkey	11.00	11.8
Egypt	8.10	8.5

Source: UN Food & Agriculture Organisation (FAO) 2013 and 2015-2016. Retrieved June2016.

Global production of tomato in the year 2014 was 170750767 tonnes from 5023810 ha of cultivated area with yield of 339883 hg/ha. The tomato production statistics for the year 2007 to 2014 for UK are shown in Table 1.3 (FAOSTAT, 2013 & 2015-16)[1].

Table 1.3: Tomato Production statistics United Kingdom 2007-2014

Year	2007	2008	2009	2010	2011	2012	2013	2014
Area harvested (ha)	213	216	213	217	216	204	220	232
Production (tonnes)	85600	88690	86800	89320	89800	83000	91100	98500
Yield (hg/ha)	4018779	4106019	4075117	4116129	4157407	4068627	4140909	4245690

Source: FAO Database, FAO Statistics Division, Release 2013 & 2014 (Updated: 16 January 2015-2016)
The area harvested is shown in hectares (ha), yield/productivity in hectogram per hectare (hg/ha) and production is in tonnes. (1 hg=100g=0.1kg and 1 metric tons or tonnes=1000kg)

Tomato is an herbaceous perennial, but is usually grown as an annual in the temperate region. The plant habit is of two types determinate and indeterminate. In case of determinate tomatoes there are 2-3 nodes between inflorescence with the

stem terminating in an inflorescence, producing fruits that are easily harvested, whereas, indeterminate plants continuously produce 3 nodes between each inflorescence, producing side shoots which terminate with fruit clusters. Tomato plant vines are typically pubescent, covered with tiny hairs.

1.2 Fruit Ripening in Tomato

Fruit ripening is defined as a developmental process during which the fruit attains an optimal eating quality. Fruit ripening and ripening associated changes in tomato are clearly marked with the change in colour, texture, flavour and aroma. Fruit ripening is altogether a very complex process recruiting several enzymes. Fruit ripening changes associated with the colour are resultant of a slow change from green to red which is due to the change of chloroplast into the chromoplast with accumulation of the pigments *viz*. lycopene and β-carotene (Grierson and Kader, 1986; Yoo *et al*., 2017). The climacteric fruits show increase in respiration and burst of ethylene ***e.g.*** Tomato, Apple, Avocado and Banana, whereas, in non-climacteric fruits there is no increase in the respiration and ethylene production.

The tomato fruit has been classified as a climacteric fruit. The respiration process in the fruit passes through a minimum level before it starts to increase during the ripening process where the respiration reaches to a maximum and then eventually declines. The respiratory peak is preceded by a marked rise in the production of ethylene (Biale and Young, 1981; Lin *et al*., 2009).

Ethylene is a gaseous hormone which plays an important role in fruit ripening. The process of ethylene synthesis is shown in fig1.1. Methionine is converted to S-adenosyl-L-methionine (SAM) by S-adenosyl-L-methioninesynthetase. The cyclic non-protein amino acid 1-aminocyclopropane and 1-carboxylic acid (ACC) is synthesized from SAM by ACC synthase (ACS) (Sato and Theologis, 1989). SAM and ACC acts as precursor of ethylene synthesis (Yang & Hoffman, 1984). ACC Oxidase (ACO) (Hamilton, *et al*., 1991) converts ACC to ethylene which is oxygen dependent and in the presence of Fe_2^+ ascorbate as co-factor (Kende, 1993, Wang *et al*., 2002). CO_2 is released as by product and HCN is also produced which is toxic and is then detoxified by β-cyanoalanine synthase producing β-cyanoalanine. ACS produces 5-methylthioadenosine (MTA) which is used for synthesis of new methionine (Lin and Grierson, 2010; Grierson, 2014).

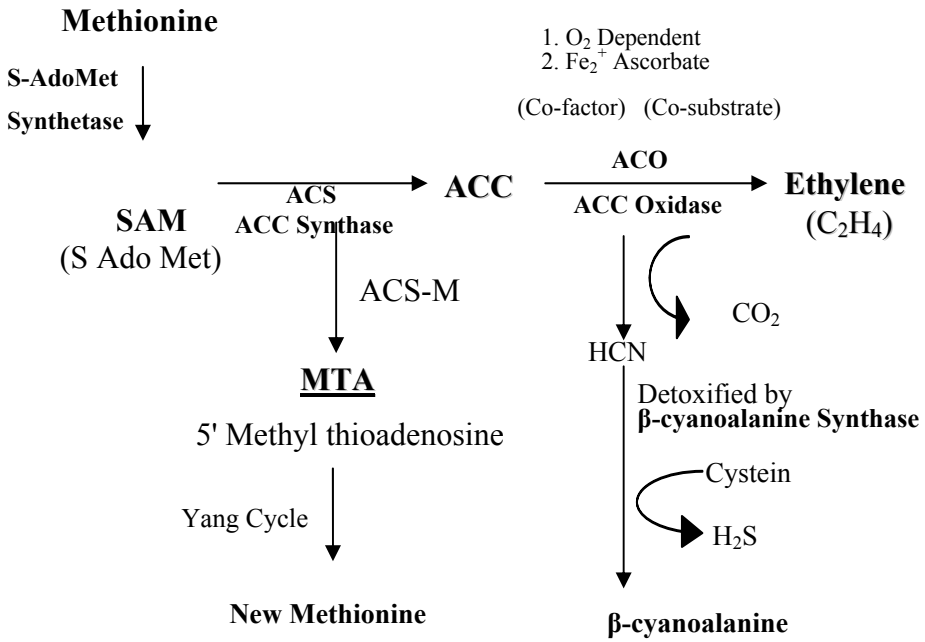


Fig. 1.1 Schematic diagram of Ethylene Synthesis

Methionine is converted into SAM by SAM synthetase. ACC is formed from SAM by ACC synthase (ACS). ACO (ACC Oxidase) converts ACC to Ethylene. ACS produces MTA, which is the by product generated during ACC synthesis. New methionine is produced from MTA via Yang cycle (redrawn from Yang & Hoffman, 1984; Wang *et al*., 2002).

There are two main system of ethylene production. System-I is active during normal vegetative growth of the plant, which is auto inhibited by ethylene. System-I is also responsible for producing basal ethylene levels by all plant tissues, including non-climacteric fruits. System-II comes into play during ripening of climacteric fruit and during petal senescence. In tomato System-I ethylene is regulated by Le ACS1A and Le ACS6 (Barry *et al*., 2000), whereas system-II is governed by Le ACS2 which in turn is regulated by Le MADS-RIN (Vrebalov *et al*., 2002; Sobolev *et al*., 2014). A number of important mutants in tomato have been identified which have helped to advance our understanding of tomato fruit ripening regulation. These include *never ripe* (*Nr*) (Rick 1956; Lanahan *et al*., 1994), *non-ripening* (*nor*) (Tigcheler, 1973), ripening-inhibitior (*rin*) (Vrebalov *et al*., 2002; Manning *et al*., 2006; Martel *et al*., 2011), *colourless non-ripening* (*cnr*) (Thompson *et al*., 1999), *green-ripe* (*Gr*) (Barry and Giovannoni, 2006), *green flesh* (*gf*) (Barry *et al*., 2008), *high pigment1* (*hp1*) and *high pigment2* (*hp2*) (Mustilli *et al*., 1999). Some of the recent studies on ethylene are listed in table1.4.1.

Table 1.4.1 Summary of recent studies on Ethylene.

Ethylene		
Genes/About	**Remarks**	**References**
Ethylene	Recent advances in ethylene research	Lin *et al.*,2009
Ethylene	New perspective in ethylene signalling	Lin, Z. and Grierson, D. 2010
Ethylene	Ethylene signaling: simple ligand, complex regulation.	Merchante *et al.*, 2013
	Role of plant hormones and their interplay in development and ripening of fleshy fruits	Kumar *et al.*,2014
LeACS	Homozygous transgenic tomato genotype (2AS-AS) that exhibits reduced ethylene production as a result of impaired expression of *LeACS2* gene by its antisense RNA and had a longer shelf life.	Sobolev *et al.*, 2014
Ethylene	Conservation of ethylene as a plant hormone over 450 million years of evolution.	Ju *et al.*,2015
Ethylene	Ethylene control of fruit ripening: revisiting the complex network of transcriptional regulation.	Liu *et al.*,2015a
Ethylene	Plant life without ethylene	Voesenek *et al.*, 2015
Ethylene	Ethylene: traffic controller on hormonal crossroads to defense	Broekgaarden *et al.*, 2015
ACS4, NR, SlETR4,SlEIL1 SlEIL3,SlERF2	Alteration of ethylene production correlated with altered ACS4 expression and enhanced the expression *NR*, *SlETR4*, *SlEIL1*, *SlEIL3*, and *SlERF2*	Sun *et al.*,2015.
Ethylene	Ethylene's role in plant mineral nutrition.	Romera *et al.*, 2016
Ethylene	Ethylene and metal stress	Keunen *et al.*, 2016
EIN2	Relaying the ethylene signal: new roles for EIN2	Zheng and Zhu, 2016
Ethylene and ABA	Comprehensive analysis of ABA effects on ethylene biosynthesis and signaling during tomato fruit ripening.	Mou *et al.*,2016
Ethylene SlETR1 & 2	Favorable effects of the weak ethylene receptor mutation *Sletr1-2* on postharvest fruit quality changes in tomatoes.	Mubarok *et al.*, 2016
Ethylene	Ethylene is involved in the actin cytoskeleton rearrangement during the root, gravitropic response of *Arabidopsis thaliana*.	Pozhvanov *et al.*, 2016
ERF	Comprehensive profiling of ERF expression identifies ripening-associated ERF genes and their link to key regulators of fruit ripening in tomato.	Liu *et al.*,2016c
Ethylene	How do plants respond to ethylene and what is its importance	Chang, 2016
Ethylene & Cytokinin	An Ethylene-induced regulatory module delays flower senescence by regulating cytokinin content.	Lin *et al.*,2017
Apetala2/ERF	Regulation of Apetala2/Ethylene Response factors in plants.	Phukan *et al.*, 2017
ACO	Aminocyclopropane-1-Carboxylate Oxidase induction in tomato flower pedicel phloem and abscission related processes are differentially sensitive to ethylene.	Chersicola, 2017
Ethylene	Ethylene regulation of fruit softening and cell wall disassembly in *Charentais melon*	Nishiyama *et al.*, 2017
Apple, ERF, MdMYC2, Jasmonate	The Jasmonate-Activated Transcription Factor MdMYC2 Regulates ERF and ethylene biosynthetic genes during Apple Fruit Ripening	Li *et al.*, 2017c

Table 1.4.2 lists the classic studies on tomato fruit ripening related genes and some mutants and table 1.4.3 shows summary of the recent work (2012-2017) on tomato fruit development, ripening, softening related genes, inflorescence and branching.

Table 1.4.2 Tomato fruit ripening related genes.

Protein	Details	References
PG PG1a, PG2a, PG2b β-subunit	Polygalacturonase PG β-subunit	Tucker and Grierson, 1982 Zeng *et al*., 1992
PE, PME	Pectin methyl esterase	Koch and Nevins, 1989, Tieman, 1992, Phan *et al*., 2007
LeACS1A,LeACS6, LeACS2,LeACS4	ACC Synthase	Barry *et al*., 2000 Alexander & Grierson, 2002
Le ACO1,3	ACC Oxidase	Barry *et al*., 1996, 2000 Chung et al., 2010
LeETR LeETR1-7 LeETR1, LeETR2 LeETR4 LeETR5	Ethylene receptor (6) Ethylene perception and signal transduction pathway. Negative regulator of ethylene signalling.	Klee 2002, Klee and Giovannoni, 2011, Wilkinson *et al.,* 1995 Yen *et al.,* 1995 Hackett *et al*., 2000 Tieman *et al*., 2000 Ciardi *et al*., 2000 Zhou *et al*., 1996a,b Lashbrook, 1998, Tieman and Klee, 1999
Le EIN3-like (EIL)	Ethylene insensitive like.	Tieman *et al*., 2001 Yokotani, 2003, 2009 Li *et al*., 2011a
Le CTR LeCTR1-4	Constitutive Triple Response	Liu *et al*., 2002, Adams-Phillips *et al.*, 2004.
Le ERF 1-6	Ethylene response factor , 4 - induction in response to Ethylene.	Tournier *et al*., 2003 Lee *et al*., 2011a
LeAGP LeEXP :LeEXP1, LeEXP2, Cel7	Arabinogalactan Expansin	Sun *et al*., 2004a,b Rose *et al*., 2000 Catala, 2000
ACO1	Aconitase 1	Hamilton *et al*., 1991
PSY1	Phytoene synthase 1	Ray *et al*., 1992
CEL1 CEL2	EGase/Cellulase; Altered pedicle and fruit but did not influence fruit softening	Lashbrook *et al*., 1998 Brummell *et al*., 1999a
TBG4	Tomato β-galactosidase - Modest impact on fruit softening in Antisense TBG4.	Smith *et al*., 2002
NR	Never Ripe	Wilkinson *et al*., 1995 Rick & Butler, 1956, Martel *et al.*, 2011
Hp Hp1, Hp2	High pigment mutants Increase green fruit and leaf chlorophyll	Peters, 1989 Mustilli *et al*., 1999
Gr	Green ripe - Gr/Nr2	Barry *et al*., 2005, Barry and Giovannoni, 2006
Gf	Gren Flesh	Barry *et al*., 2008
LeMADS-RIN	MADS box transcriptional factor (*rin* loci)	Vrebalov *et al*., 2002, 2009 Seymour et al., 2011
SlMYB2	MYB Transcription Factors	Adato *et a*l., 2009
AP1 AP2	Member of ERF Superfamily APETALA	Karlova *et al*., 2011 Chung *et al.,* 2010 Kwon *et al*., 2011
LeTAGL	*TOMATO AGAMOUS-LIKE 1* (*TAGL1*) Pleiotropic phenotype	Vrebalov *et al*., 2009 Itkins et al., 2009
LeNOR	Non Ripening, Signalling, ripening in climacteric fruits	Adamphillip *et al*., 2004

LeSitw Sitiens	Over ripe fruit of ABA deficiency mutant → precocious germination	Liu *et al*., 1996
Brassinosteroids (BRs)	Polyhydroxysteroids phytohormones Exogenous application of BR to tomato pericarp elevated the	Bishop, 2003
	• level of lycopene • Lower level of chlorophyll • Decreased ascorbic acid • Increased carbohydrate content • Brassinosteroid deficiency	Xardhini and Rao, 2002
d^x	Extreme dwarf; Brassinosteroid deficient	Lisso and Alt, 2003
dpy	• Delayed ripening • Reduced carbohydrate • Decreased dry weight • Lower acid invertase • Low fruit yield Dumpy Brassinosteroid insensitive signalling mutant	
Jasmonic acid (JA) Methyl jasmonate (MeJA)	Derivation of linolenic acid - modulates aspects of fruit ripening, pollen variability, root growth.	Creelman and Mullet, 1997
Polyamines (PA) Free Polyamines	Low mol. Wt. organic cations. Affect large no. of development and physiological responses. • Acts as anti-senescence agents • Retarded fruit colour change. • Increased fruit firmness • Delayed ethylene and respiration rate emission. • Induced mechanical stress resistance	Walter, 2003 Valero *et al*., 2002
Tomato ODC (Ornithine decarboxylase)	Acts for polyamine synthesis	Alabadi and Carbinell, 1998
ADC (Arginine decarboxylase)	Cell expression and division during early fruit growth	Cohn *et al*., 1982
Kinase LeSNF1 LeSNF4	Regulation SNF4; substrate of SnRK1 Influenced by ABA and GA	Bradford *et al*., 2003
SnRK	Sucrose non-fermenting related kinase 1 ABA - Sugar sensing	Himmelbach *et al*., 2003 Lunn and MacRae, 2003
LeFRK2	Tomato fructokinase	Dai, 2002
Lin	Tomato invertase - Lin 5,6,7,8	Godt and Rolster 1997
CDKA1 ; CDKA2	Cyclin dependent kinase	Joubes *et al*., 1999
DR12 DR1,3,4,8	Auxin response factor	Jones *et al*., 2002
Dgt LeIAA2,8	Digeotropica Blocked Auxin	Zobel (1972) Balbi Lomel, 2003
LeSPH1 GAD3	Le S- Protein Homologue glutamate decarboxylase isoform3	Testa *et al*., 2002
LeH2A2	Similar to histone H2A, Responds to GA3	van den Heuvel *et al*., 1999
pat Pat-2 Pat3/pat4	Parthenocarpy Auxin 3 times higher GA 4 times higher	Fos *et al*., 2001 George *et al*., 1984 Nuez *et al*., 1986
Gib-1 Gib-2	GA deficient mutants	Groot *et al*., 1987 Bensen and Zeevart, 1990
LeCPS	Le20OX-1,-2 copalyl diphosphate synthase	Rebers *et al*., 1999

ful	FUL1 (Previous Sl TDR4) FUL2 (Previous Sl MBP7)	Bemer *et al*., 2012 Mintz-Oron *et al*., 2008
polycotyledon mutant	The *polycotyledon mutant* of tomato shows enhanced polar auxin transport	Al-Hammadi, *et al.*,2003
polycotyledon pct1-2	The *polycotyledon* (*pct1-2*) mutant of tomato shows enhanced accumulation of PIN1 auxin transport facilitator protein.	Kharshiing *et al.*,2010

Table 1.4.3 Summary of tomato fruit development, ripening, softening related genes, inflorescence and branching.

Tomato		
Genes/About	**Remarks**	**References**
TED database	Tomato Expression Database (TED): a suite of data presentation and analysis tools	Fei *et al.*,2006
Tomato FT	Universal florigenic signals triggered by FT homologues regulate growth and flowering cycles in perennial day-neutral tomato.	Lifschitz and Eshed,2006
Tomato FT	The tomato FT ortholog triggers systemic signals that regulate growth and flowering and substitute for diverse environmental stimuli	Lifschitz *et al.*, 2006
Flowering time tomato	Characterization of tomato mutants affected in their flowering time and in the morphogenesis of their reproductive structure	Quinet *et al.*,2006
LANCEOLATE & miR319	Regulation of LANCEOLATE by miR319 is required for compound-leaf development in tomato	Ori *et al.*,2007
inflorescence in tomato	The making of a compound inflorescence in tomato and related nightshades.	Lippman *et al.*, 2008
Leaf developmetn	A protracted and dynamic maturation schedule underlies Arabidopsis leaf development	Efroni *et al.*,2008
FASCICULATE-Selfpruning in peppers	Co-ordinated regulation of flowering time, plant architecture and growth by FASCICULATE: the pepper orthologue of SELF PRUNING.	Elitzur *et al.*,2009
GOBLET	The NAC-domain transcription factor GOBLET specifies leaflet boundaries in compound tomato leaves	Berger *et al.*,2009
FLORIGEN	The flowering hormone florigen functions as a general systemic regulator of growth and termination	Shalit *et al.*,2009
SHATTER PROOF gene TAGL1	Fleshy fruit expansion and ripening are regulated by the Tomato SHATTERPROOF gene TAGL1	Vrebalov *et al.*, 2009
Leaves	Morphogenesis of simple and compound leaves	Efroni *et al.*,2010
OrrDS	The *Orange ripening* (*OrrDS*) tomato mutant	Nashilevitz *et al.*, 2010
RAB and ROP in Grapes	Role of ROP and RAB GTPases in grape berry development	Sauvage *et al*., 2010
Small GTPases in Peach	Analysis of Small GTPases in Peach during fruit development and ripening	Falchi *et al*.,2010
TFGD database	Tomato Functional Genomics Database: a comprehensive resource and analysis package for tomato functional genomics.	Fei *et al.*,2011
Peach melting and non melting flesh and endo-PG	A comparative study of melting and non-melting flesh peach cultivars reveals that during fruit ripening endo-polygalacturonase (endo-PG) is mainly involved in pericarp textural changes and not in firmness reduction	Ghiani *et al.*,2011
Tomato fruit ripening	Genetics and control of tomato fruit ripening and quality attributes.	Klee and Giovannoni, 2011
RIN and CNR	The tomato MADS-box transcription factor RIPENING INHIBITOR interacts with promoters involved in numerous ripening processes in a COLORLESS NONRIPENING-dependent manner.	Martel *et al.*,2011

Peach fruit ripening	Peach fruit ripening: A proteomic comparative analysis of the mesocarp of two cultivars with different flesh firmness at two ripening stages	Prinsi *et al.*,2011
Co-expression and clustering	Exploring tomato gene functions based on coexpression modules using graph clustering and differential coexpression approaches.	Fukushima *et al.*, 2012
l1 and *l2*	*lutescent 1* and *2* (*l1* and *l2*) mutant tomato	Barry *et al.*,2012
FRUITFULL TDR4/FUL1, MBP7/FUL2,	The tomato FRUITFULL homologs TDR4/FUL1 and MBP7/FUL2 regulate ethylene-independent aspects of fruit ripening.	Bemer *et al.*,2012
Fruit firmnessQTL	High-resolution mapping of a fruit firmness-related quantitative trait locus in tomato reveals epistatic interactions associated with a complex combinatorial locus	Chapman *et al.*,2012
Terminating flower gene	Synchronization of the flowering transition by the tomato TERMINATING FLOWER gene	MacAlister *et al.*, 2012
Tomato genome	The tomato genome	Michael and Alba, 2012
MACROCALYX (MC)	MACROCALYX (MC) gene is involved in the development of sepals in the first whorl and in inflorescence determinacy in tomato	Nakano *et al.*, 2012
Seed & fruit set	Molecular regulation of seed and fruit set.	Ruan *et al.*,2012
TCPs and cytokinin	Class I TCPs modulate cytokinin-induced branching and meristematic activity in tomato.	Steiner *et al.*, 2012
Tomato floral meristem	Repression of floral meristem fate is crucial in shaping tomato inflorescence.	Thouet *et al.*, 2012
Fleshy fruit evolution	The tomato genome sequence provides insights into fleshy fruit evolution	Tomato Genome Consortium, 2012
ARF3 & 4	Failure of the tomato trans-acting short interfering RNA program to regulate AUXIN RESPONSE FACTOR3 and ARF4 underlies the wiry leaf syndrome	Yifhar *et al.*,2012
Anthocyanin *Aft/Aft atv/atv*	Accumulation of anthocyanins in *Aft/Aft atv/atv* tomato skin extends shelf life and are less susceptible to *Botrytis cinerea*	Bassolino *et al.*, 2013
Florigen	Tomato yield heterosis is triggered by a dosage sensitivity of the florigen pathway that fine-tunes shoot architecture.	Jiang *et al.*,2013
Transcriptome profiles	Dynamic transcriptomic profiles between tomato and a wild relative reflect distinct developmental architectures	Chitwood *et al.*, 2013
SlMADS1	A tomato MADS-box transcription factor, SlMADS1, acts as a negative regulator of fruit ripening.	Dong *et al.*,2013
Cytokinin and leaf maturation	Regulation of leaf maturation by chromatin-mediated modulation of cytokinin responses	Efroni *et al.*,2013
Cytokinin	A cytokinin-activating enzyme promotes tuber formation in tomato.	Eviatar-Ribak *et al.*, 2013
RIN and tomato fruit ripening	A large-scale identification of direct targets of the tomato MADS box transcription factor RIPENING INHIBITOR reveals the regulation of fruit ripening.	Fujisawa *et al.*,2013
ETTIN (ARF3)	Dual regulation of ETTIN (ARF3) gene expression by AS1-AS2, which maintains the DNA methylation level, is involved in stabilization of leaf adaxial-abaxial partitioning in Arabidopsis	Iwasaki *et al.*, 2013
MiRNA in fruit development	Identification of microRNA targets in tomato fruit development using high-throughput sequencing and degradome analysis.	Karlova *et al.*, 2013
Flavour	Genetic challenges of flavor improvement in tomato	Klee and Tieman, 2013
Tomato ILs	Metabolic engineering of tomato fruit organic acid content guided by biochemical analysis of an introgression line	Morgan *et al.*, 2013
NAC and Ethylene	NAC transcription factor controls ethylene-regulated cell expansion in flower petals.	Pei *et al.*,2013
SlARF4	SlARF4, an auxin response factor involved in the control of sugar metabolism during tomato fruit development.	Sagar *et al.*,2013

Tomato	Fruit development and ripening	Seymour *et al.*, 2013
SlERF36	SlERF36, an EAR-motif-containing ERF gene from tomato, alters stomatal density and modulates photosynthesis and growth.	Upadhyay *et al.*, 2013
Ethylene and Auxin	Coordination of auxin and ethylene biosynthesis by the aminotransferase VAS1.	Zheng *et al.*,2013
Florigen	Florigen and anti-florigen - a systemic mechanism for coordinating growth and termination in flowering plants	Lifschitz *et al.*, 2014
Florigen	Optimization of crop productivity in tomato using induced mutations in the florigen pathway.	Park *et al.*,2014b
Gene Network inflorescence	Inferring the Gene Network Underlying the Branching of Tomato Inflorescence	Astola *et al.*,2014
FRUITFULL, SVP SOC1	Sequential action of FRUITFULL as a modulator of the activity of the floral regulators SVP and SOC1	Balanzà *et al.*,2014
Fruit ripening	Metabolic differences in ripening of Ailsa Craig tomato and three monogenic mutants.	Beisken *et al.*,2014
Senescence	Molecular programme of senescence in dry and fleshy fruits.	Gómez *et al.*,2014
TOPLESS	Genome-wide identification, phylogenetic analysis, expression profiling, and protein-protein interaction properties of TOPLESS gene family members in tomato.	Hao *et al.*,2014
Tomato	Genome to phenome in tomato	Ichihashi and Sinha, 2014
SGN	The Sol Genomics Network (SGN)--from genotype to phenotype to breeding.	Fernandez-Pozo *et al.*,2014
Genomics of tomato	Functional genomics of tomato: Opportunities and challenges in post-genome NGS era.	Kumar and Khurana, 2014
SlNAC1	Overexpression of tomato SlNAC1 transcription factor alters fruit pigmentation and softening.	Ma *et al.*,2014
Flavonols	The anthocyanin reduced tomato mutant demonstrates the role of flavonols in tomato lateral root and root hair development.	Maloney *et al.*,2014
Plum glossiness	Non-invasive assessment of glossiness and polishing of the waxbloom of European plum	Mukhtar *et al.*,2014
Ap2/ERF-SlERF52	The AP2/ERF transcription factor SlERF52 functions in flower pedicel abscission in tomato.	Nakano *et al.*, 2014
Meristem maturation	Meristem maturation and inflorescence architecture--lessons from the Solanaceae.	Park *et al.*,2014a
Florigen	Optimization of crop productivity in tomato using induced mutations in the florigen pathway.	Park *et al.*,2014
Inflorescence development	Inflorescence development in tomato: gene functions within a zigzag model.	Périlleux, *et al.*, 2014
TAP3,TM6	A mutation in TAP3 and the silencing of TM6 both result in the conversion of stamens into carpels and a more or less severe conversion of petals into sepals	Quinet *et al.*, 2014
Tomato genomic database	Tomato genomic resources database: an integrated repository of useful tomato genomic information for basic and applied research.	Suresh *et al.*,2014
Metabolism during fruit development	Regulation and function of secondary metabolism during fruit development and ripening	Tohge *et al.*,2014
FRUITFULL	Members of the tomato FRUITFULL MADS-box family regulate style abscission and fruit ripening.	Wang *et al.*,2014a
SlFYFL	overexpression of the MADS-box gene SlFYFL delays senescence, fruit ripening and abscission in tomatoes	Xie *et al*., 2014
DELLA and GA	Uncovering DELLA-Independent Gibberellin Responses by characterizing new tomato *procera Mutants*.	Livne *et al.*,2015
CIN1	Ectopic overexpression of the cell wall invertase gene CIN1 leads to dehydration avoidance in tomato.	Albacete *et al.*, 2015
FGR genes	Fruit growth-related genes in tomato	Azzi *et al.*,2015

Chromomethylase3 in CNR tomato	Requirement of CHROMOMETHYLASE3 for somatic inheritance of the spontaneous tomato epimutation Colourless non-ripening. (CNR)	Chen *et al.*,2015
Network Analyses Sun tomato	Network Analyses Reveal Shifts in Transcript Profiles and Metabolites That Accompany the Expression of SUN and an Elongated Tomato Fruit.	Clevenger *et al.*, 2015
Tomato as model crop	Tomato as a model crop in biotechnology	Gerszberg *et al.*, 2015
SlARF2	Auxin Response Factor SlARF2 Is an Essential Component of the regulatory mechanism controlling fruit ripening in tomato.	Hao *et al.*,2015
Abscission	Development and regulation of pedicel abscission in tomato.	Ito and Nakano, 2015
MIXTA	Tomato MIXTA-Like transcription factor coordinates fruit epidermis conical cell development and cuticular lipid biosynthesis and assembly	Lashbrooke *et al.*, 2015
KNOTTED1-LIKE HOMEOBOX	A KNOTTED1-LIKE HOMEOBOX protein regulates abscission in tomato by modulating the auxin pathway.	Ma *et al.*,2015
Chemical sensors	Non-destructive tomato ripeness determination using carbon nanostructure based chemical sensors. application of tristimulus and princial component analysis methods	Márcia *et al.*,2015
APETALA1 FRUITFULL	Functional Divergence of APETALA1 and FRUITFULL is due to changes in both regulation and coding sequence.	McCarthy *et al.*, 2015
SVP, MADS, Jointless	Apple SVP family MADS-box proteins and the tomato pedicel abscission zone regulator JOINTLESS have similar molecular activities	Nakano *et al.*, 2015
Ripening	Ripening genetics of the tomato fruit.	Omboki *et al.*, 2015
Tomato fruit evaluation	Non-destructive techniques for evaluating quality of fruits	Ravindran *et al.*, 2015
Fruit morphology	Evolutionary developmental genetics of fruit morphological variation within the Solanaceae.	Wang *et al.*,2015b
Lycopene	A dramatic effect of oxygen on protection of human cells against c-radiation by lycopene.	Boehm *et al.*,2016
SlTIR1	SlTIR1 is involved in crosstalk of phytohormones, regulates auxin-induced root growth and stimulates stenospermocarpic fruit formation in Micro-Tom and Ailsa Craig tomato	Ren and Wang, 2016
Texture peppers	Evaluation of mineral profile, texture, sensory and structural characteristics of old pepper landraces.	Ilic *et al.*,2016
Meristem	Active suppression of a leaf meristem orchestrates determinate leaf growth	Alvarez *et al.*, 2016
SlGA2	Identification and characterization of tomato gibberellin 2-oxidases (GA2oxs) and effects of fruit-specific SlGA2ox1 overexpression on fruit and seed growth and development	Chen *et al.*,2016
SlGID2	Silencing SlGID2, a putative F-box protein gene, generates a dwarf plant with dark-green leaves in tomato.	Liu *et al.*,2016d
Pectin	Pectin biosynthesis, trafficking and dynamics	Anderson *et al.*, 2016
Networks and fruit ripening	*In silico* transcriptional regulatory networks involved in tomato fruit ripening.	Arhondakis *et al.*, 2016
Antioxidants	Exploiting genomics resources to identify candidate genes underlying antioxidants content in tomato fruit	Calafiore *et al.*, 2016
Fruit types & Climate	Plants show more flesh in the tropics: variation in fruit type along latitudinal and climatic gradients.	Chen *et al.*,2017
Peach flesh softening	A proposal for determining the flesh softening of peach and nectarinein postharvest through simplified targeted modelling	Contador *et al.*,2016
Tomato and Peach fruits and leaves	Phosphoenolpyruvate carboxykinase, pyruvate orthophosphate dikinase and isocitrate lyase in both tomato fruits and leaves, and in the flesh of peach and some other fruits.	Famiani *et al.*,2016

Lycopene	Dissection of tomato lycopene biosynthesis through VIGS	Fantini *et al.*,2016
Fruit metabolite networks	Fruit metabolite networks in engineered and non-engineered tomato genotypes reveal fluidity in a hormone and agroecosystem specific manner.	Fatima *et al.*,2016
Tomato seeds	Genomic imprinting in the endosperm is systematically perturbed in abortive hybrid tomato seeds.	Florez-Rueda *et al.,* 2016
Chromatin regulation	DNA methylation and chromatin regulation during fleshy fruit development and ripening.	Gallusci *et al.,* 2016
AGAMOUS1 ARLEQUIN	TOMATO AGAMOUS1 and ARLEQUIN/TOMATO AGAMOUS-LIKE1 MADS-box genes have redundant and divergent functions required for tomato reproductive development	Gimenez *et al.*,2016
Antisense tomato	Identifying and silencing tomato ripening genes with antisense genes	Grierson, 2016
Tomato as model crop	Tomato Fruits as model for metabolic engineering of terpenes	Gutensohn and Dudareva, 2016
TPI- and SlGLO1	TPI- and SlGLO1-silenced plants exhibit aberrant carpelloid stamens in tomato	Guo *et al*., 2016
Hairless gene, actin, trichomes	Molecular cloning of the tomato Hairless gene implicates actin dynamics in trichome-mediated defense and mechanical properties of stem tissue	Kang *et al.*,2016
RNA-Seq Analysis	Comprehensive RNA-Seq analysis on the regulation of tomato ripening by exogenous auxin	Li *et al.*,2016b
SlMSI1	Polycomb-group protein SlMSI1 represses the expression of fruit-ripening genes to prolong shelf life in tomato.	Liu *et al.*,2016
Carotenoid	Tomato fruit carotenoid biosynthesis is adjusted to actual ripening progression by a light-dependent mechanism	Llorente *et al.*,2016
Carotenoid and polyphenols	Tomato as a source of carotenoids and polyphenols targeted to cancer prevention.	Martí *et al.*,2016
SlNAC1	Suppression of tomato SlNAC1 transcription factor delays fruit ripening.	Meng *et al.*,2016
carotenoid-deficient mutants	Fruit carotenoid-deficient mutants in tomato reveal a function of the plastidial isopentenyl diphosphate isomerase (IDI1) in carotenoid biosynthesis.	Pankratov *et al.,* 2016
eQTLs tomato	eQTL regulating transcript levels associated with diverse biological processes in tomato	Ranjan *et al.*,2016
ABA network in tomato	Integrated bioinformatics to decipher the ascorbic acid metabolic network in tomato	Ruggieri *et al.,* 2016
Tomato fruit evaluation	Color, size, volume, shape and texture feature extraction techniques for fruits	Satpute and Jagdale, 2016
Tomato fruit softening	Genetic improvement of tomato by targeted control of fruit softening	Uluisik *et al.,* 2016
Tomato Organ Abscission.	De novo transcriptome sequencing and development of abscission zone-specific microarray as a new molecular tool for analysis of tomato organ abscission.	Sundaresan *et al.,* 2016
Plum fruit ripening and development	Patterns of flesh reddening, translucency, ethylene production and storability of 'Friar' plum fruit harvested at three maturity stages as affected by the storage temperature.	Wang *et al.*,2016
SlZFP2	RNA sequencing analysis of gene expression regulated by the transcription factor *SlZFP2* during early fruit development,	Weng *et al.*,2016
APETALA2/ERF	Roles of APETALA2/ Ethylene-Response Factors in regulation of fruit quality	Xie *et al.*,2016
Pear and Rosaceae genomics	Genomics of pear and other Rosaceae fruit trees.	Yamamoto and Terakami, 2016
Apetala1/Fruitfull, Sepallata, Agamous-Like6	Prevalent Exon-Intron Structural Changes in the APETALA1/FRUITFULL, SEPALLATA, AGAMOUS-LIKE6, and FLOWERING LOCUS C MADS-Box gene subfamilies provide new insights into their evolution	Yu *et al.*,2016

Pear fruit transcriptome	Distinct transcriptome profiles reveal gene expression patterns during fruit development and maturation in five cultivated species of pear	Zhang *et al.*, 2016b
Tomato fruit transcriptome	Spatiotemporal transcriptome provides insights into early fruit development of tomato	Zhang *et al.*, 2016c
KRP	Coordination of meristem doming and the floral transition by late termination, a kelch repeat protein	Tal *et al.*,2017
Sl*AGAMOUS-LIKE 6*	Tomato facultative parthenocarpy results from Sl*AGAMOUS-LIKE 6* loss of function.	Klap *et al.*,2017
Peach SEP1 SEPALLATA	Characteristics and regulatory pathway of the PrupeSEP1 SEPALLATA gene during ripening and softening in peach fruits.	Li *et al.*,2017
Auxin	Constitutive Expression of Arabidopsis SMALL AUXIN UP RNA19 (SAUR19) in tomato confers auxin-independent hypocotyl elongation.	Spartz *et al.*, 2017
MS and pico probe	Blossom end rot tomato fruit diagnosis for *in situ* cell analyses with real time pico-pressure probe ionization mass spectrometry	Gholipour *et al.*, 2017
Peach fruit softening	Monitoring the flesh softening and the ripening of peach during the last phase of growth on-tree.	Pinto *et al.*,2017
SlMBP11	The MADS-box gene SlMBP11 regulates plant architecture and affects reproductive development in tomato plants.	Guo *et al.*,2017
Leafless	Coordination of auxin-triggered leaf initiation by tomato LEAFLESS	Capua and Eshed, 2017
Rosaceae phylogeny	Evolution of rosaceae fruit types based on nuclear phylogeny in the context of geological times and genome duplication	Xiang *et al.*,2017
Apple ripening	Evaluation of the ripening stages of apple by means of computer vision system	Cardena-Perez *et al.*, 2017
Gene expression	Flexible tools for gene expression and silencing in tomato	Fernandez *et al.*, 2017
Pectin and hemicelulose	Remodeling of pectin and hemicelluloses in tomato pericarp during fruit growth.	Guillon *et al.*, 2017
Tomato shoot regenration	Metabolomic homeostasis shifts after callus formation and shoot regeneration in tomato.	Kumari *et al.*, 2017
Library	*In silico* screening of a saturated mutation library of tomato	Menda *et al.*,2017
Diversity in tomato	Domestication rewired gene expression and nucleotide diversity patterns in tomato.	Sauvage *et al.*, 2017
Tomato flavor	A chemical genetic roadmap to improved tomato flavor.	Tieman *et al.*, 2017
SlUGT75C1	Suppressing ABA uridine diphosphate glucosyltransferase (SlUGT75C1) alters fruit ripening and the stress response in tomato	Sun *et al.*,2017
Tomato flavor	Tomato Flavor: Lost and Found	Wang and Seymour, 2017
SlVPE3	Post-transcriptional regulation of fruit ripening and disease resistance in tomato by the vacuolar protease SlVPE3	Wang *et al.*,2017b
Eocene lantern fruits	Eocene lantern fruits from Gondwanan Patagonia and the early origins of Solanaceae.	Wilf *et al.*,2017
SlPL	Silencing of *SlPL* (Solyc03g111690) which encodes a Pectate Lyase in tomato, confers enhanced fruit firmness, prolonged shelf-life, and reduced susceptibility to gray mold.	Yang *et al.*,2017
Tomato fruit carotenoids	Inferring the genetic determinants of fruit colors in tomato by carotenoid profiling.	Yoo *et al.*,2017

The plant cell wall of dicots consists of cellulose, hemicellulose, pectin and protein. The cell wall undergoes considerable changes during ripening. The cell wall hydrolases play an important role in fruit ripening. Several genes are involved in cell wall degradation *eg*. polygalacturonase (PG), pectinestrase (PE), for ethylene synthesis such as ACC synthase and ACC oxidase and the genes responsible for the carotenoid synthesis such as phytoene synthase (Gray *et al*, 1994). The inhibition of these genes using antisense RNA technology for the production of transgenic tomatoes exhibit stably inherited traits useful in the modification of the fruit colour, flavour, softening and delayed ripening (Grierson and Fray, 1994). Antisense technology involves the introduction of a gene sequence in reverse orientation of the target gene. The RNA thus produced from the gene with reverse orientation is called Antisense RNA, which forms a duplex with sense RNA thus preventing the translation of Sense RNA to produce the protein. Many genes related to fruit ripening have been down regulated in transgenic tomato like PG antisense (Sheely, 1988, Smith, 1988, 1990), PE antisense (Hall *et al*., 1993), ACC Synthase (Oeller *et al*., 1991), Ethylene forming enzyme (ACC) (Hamilton *et al*., 1991) and phytoene synthase (Bird *et al*., 1991).

1.3 Plant cell wall and its components

The plant cell wall is composed of three layers: middle lamella, primary and secondary cell wall. During cell division middle lamella is formed first followed by formation of the primary cell on either side of the middle lamella and then the secondary cell wall. Plant cell walls are composed of basic building blocks. All plant cell walls are composed of cellulose microfibrils that form the mechanical framework of the wall, and a matrix phase that forms cross-links among the microfibrils and fills the space between the fibrillar frameworks (Carpita and Gibeaut, 1993; O'Neill and York, 2003; Somerville *et al*., 2004; Bashline *et al*., 2014). Cell walls are composed of a small number of distinct chemical polymers tightly woven into a meshwork allowing it to perform various mechanical and biochemical functions (Carpita and Gibeaut, 1993; Somerville *et al*., 2004; Humphrey *et al.,* 2007; Oda 2015; Yokoyama *et al*., 2016).

Cell wall consists of polysaccharides and proteins. Three main groups of polysaccharides in plant cell wall are:-

1. **Cellulose**: The content of cellulose is 20-30% of the dry weight of cell wall (Saxena and Brown, 2005). The cellulose micro fibrils are made up of β-1,4-D-glucan (Somerville, 2006; McFarlane *et al*., 2013; Schwerdt *et al*., 2015).

2. **Hemicellulose**: It makes up 20-25% of cell wall and their structural composition is highly variable. The xyloglucan forms major part of hemicellulose (Brett and Waldron, 1996). Other examples of hemicellulose are glucomannans, galactoglucomannans, arabinoxylans (Brummell and Harpster, 2001; Attia and Brummer, 2016) and also includes gluconoarabinoxylans and mixed linkage glucans (MLG) (Rui and Anderson, 2015; Fasoli *et al*., 2016; Pauly and Keegstra, 2016).

3. **Pectin:** The major polysaccharides present in the matrix of middle lamella and primary cell wall which consists of 30-40% of α-1-4 linked D-galacturonic acid (McCartney and Knox, 2002; Fasoli *et al*., 2016; Lionetti *et al*., 2017). Pectin is formed of three main components

    i. Homogalacturonan (HG)

    ii. Rhamnogalacturonan I (RG-I)

    iii. Rhamnogalacturonan II (RG-II)

HG is the most abundant in pectins, and is found in middle lamella and primary cell wall (Zhan *et al*., 1998). The other component that forms pectin is xylogalacturonan (Willats *et al.,* 2001; Mercadante *et al.,* 2013, 2014; Rui and Anderson, 2015).

Apart from polysaccharides present in cell wall there are 5-10% of different cell wall proteins which are either structural proteins or enzymes. The cell wall proteins usually present are glycoproteins which include hydroxyproline rich glycoprotein, proline rich proteins and glycine rich proteins (Cassab, 1998). However, it has also become clear that not all cell walls have exactly the same chemical composition and probably exhibit different designs. The non-grass angiosperms have high amount of hemicelluloses (fucoside xyloglucan (XG), xylans, some mannans) and structural proteins. The primary walls have high amounts of pectins (homogalacturonans (HG), rhamnogalacturonan (RG I and II) while the secondary walls have high amounts of lignins (Sarkar *et al*, 2009). The cell wall composition

of gymnosperm is similar to non-grass angiosperms except they have higher amount of glucomannans and their lignins are homogeneous consisting primarily of guaicyl units. The macromolecular composition of the cell wall has characteristically evolved differently in different plants (Sarkar *et al*, 2009; Li *et al*., 2015).

1.4 Fruit softening and cell wall modification

Fruit ripening is a complex trait; the fleshy fruits undergo several physiological changes including the change in colour, flavour and texture. As the ripening process progresses the fruit undergoes marked softening to attain an edible texture. Genetic engineering has been used to characterize the different pathways involved in the fruit ripening associated changes, including fruit softening in order to alter the fruit quality through change in colour, aroma and firmness (Saladie *et al.*, 2007). There are several factors associated with the fruit softening which affect fruit firmness as the fruit undergoes several changes from mature green to red ripe stage (Seymour *et al.,* 2013, Ng *et al*., 2015).

Research spanning more than forty years has targeted the mechanism of fruit softening which coincides with the dissolution of the middle lamella resulting in the reduction in intercellular adhesion, depolymerisation and solubilization of the hemicellulosic and cell wall polysaccharides (Brummell and Harpster, 2001). These events are marked by the increased expression of numerous cell wall degrading enzymes, including polysaccharide hydrolases, transglycosylases, lyases and other cell wall loosening proteins, such as expansin (Harker *et al*., 1997; Rose *et al*., 2003; Brummell, 2006).

Factors such as cellular turgor and morphology (Shackel *et al*., 1991, Seymour *et al*., 2013) have been suggested to contribute to aspects of texture; mechanistic models almost invariably attribute fruit softening to disassembly of polysaccharide networks in the primary cell wall and middle lamella (Rose *et al*., 2003; Brummell, 2006; Ng *et al*., 2015; Fasoli *et al*., 2016). A study has shown that inhibition of pectinesterase in tomato causes fruit to soften more quickly (Phan *et al*., 2007; Lunn *et al*., 2013).

The synthesis and modification of the cell wall must involve the production of new cell wall polymers and enzymes. Cell wall polymer biosynthesis occurs

mainly in the Golgi apparatus, except for cellulose, which is made at the plasma membrane. Polysaccharides are synthesised at the cell membrane or in the Golgi apparatus and secreted proteins are usually synthesised on the endoplasmic reticulum (ER) and transported via the Golgi and the *trans*-Golgi network (TGN) to the plasma membrane (Hanton and Brandizzi, 2006; De Caroli *et al*., 2011;Wordon *et al*., 2012; Zhu *et al*., 2015; van de Meene *et al.,*2017).

In tomato fruit, the use of gene silencing for the inhibition of the synthesis of PG, and reduced PE levels to as little as 1% of the normal level in wild type and inhibition of pectin depolymerisation had only a small effect upon fruit firmness (Kramer *et al*., 1992; Langley *et al*., 1994). The mode of action of the cell wall hydrolases is as follows:-

- **Polygalacturonase (PG):-** Poly (1-4-α-D-galacturinide) glycan hydrolase hydrolyses the α(1-4) links between demethylated galactouronic acid residue. These two enzymes can act synergistically with PE generating sites for PG action. Based on its action, PG is of two types: endo-polygalacturonase and exo-polygalacturonase. Exo type (EC3.2.1.67) removes single galacturonic acid units from the non reducing end of poly galacturonic acid whereas; endo type (EC3.2.1.15) cleaves such polymers at random. The substrate for PG in the cell wall is mainly homogalacturonans (HG). Which are secreted to the cell wall in a highly methyl-esterified form which must be de-esterified before they can become a substrate for PG (Jarvis, 1984, Carpita and Gibeaut, 1993; Fabi *et al*., 2014; Latarullo *et al.,*2016).
- **Pectin Methyl Esterase (PME):** removes methyl group from C-6 position of a galactouronic acid. PME de-esterifies polyuronides by removing methyl group from the C-6 position of galacturonic acid residues of Pectin resulting in the carboxyl form, change in pH and change in cell wall and makes polyuronide susceptible to degradation by PG (Pressey and Avants, 1982; Jarvis, 1984; Seymour, 1987; Koch and Nevins 1989; Carpita and Gibeaut, 1993; Mercadante *et al.,*2013, 2014; Paniagua, *et al.,*2014; Segonne *et al.,*2014; Sénéchal *et al.,*2015; Weraduwage *et al.,*2016; Lionetti *et al.,*2017; Hocq *et al*., 2017, Xu *et al*., 2017).

- **β-Galactosidase:** Exo-β-D-galactosidase (EC3.2.1.23) removes the terminal non-reducing β-D-galactosyl residues from β-D-galactosides. Total β-D-galactosidase activity is high and does not change appreciably during tomato fruit development and ripening (Carey *et al*., 1995; Yakubov *et al*., 2016; Zhang *et al*., 2017).

- **Endo(1-4)β-D-glucanase:** E-Gase (EC 3.2.1.4) hydrolyse internal linkage of (1-4)β-D-linked glucan chains adjacent to unsubstituted residues and in vitro are active against xylo-glucan, cello-oligosaccharides, non-crystalline cellulase and carboxy methyl cellulose (CMC) (Ohmiya *et al*., 1995; Attia and Brummer, 2016).

- **Xyloglucan endo transglycosylase (XET):** XET **(**EC 2.4.1.207) is also known as Endo-xyloglucan Transferase (EXGT). EXGT cleaves the internal linkage of (1-4) β-D-glucan backbone of xyloglucan and transfer the newly formed potentially reducing end to C-4 position of glucose unit at non-reducing end of another xyloglucan polymer (Smith and Fry, 1991; Simmons *et al.,* 2016; Shinohara *et al.,* 2017).

- **Expansin (EXP):** EXP probably act by causing a reversible disruption of H-bonding between cellulose microfibrills and matrix polysaccharides (xyloglucan) resulting in loosening of the wall and allow turgor driven slippage of microfibrills (Cosgrove, 2000; Whitney *et al*., 2000; Dal Santo *et al*., 2013; Minoia *et al*., 2016; Perini *et al*., 2017a,b).

- **Cellulase:** - hydrolyses the β(1-4) link between adjacent glucose residues (McFarlane *et al*., 2014; Li *et al*., 2016c).

Likewise, the inhibition or overexpression of PE (Hall *et al*., 1993) β-glucanase (Lashbrook *et al*., 1998) and xyloglucan endotransglycosylase (de Silva *et al*., 1994) had little or no effect upon fruit softening, though suppression of galactanase gene expression reduced softening by 40% (Smith *et al*., 2002) and inhibition of the ripening specific expansin reduced softening by up to 20% and increased the degree of pectin polymerisation (Brummell *et al*., 1999b). Genes involved in fruit softening, cell wall disassembly involved and associated with the textural properties of the fruit are listed in table 1.5.

Table 1.5 Recent studies on Cell wall, fruit softening and texture

Cell wall		
Genes/About	**Remarks**	**References**
Mango texture	Mango fruit ripening associated changes in cell wall constituents in relation to textural softening	Yashoda *et al.*, 2006
RAB	The role of Rab GTPases in cell wall metabolism	Lycett, 2008
PCW	Microanalysis of plant cell wall polysaccharides	Obel *et al.*,2009
Cell wall proteome	Straying off the Highway: trafficking of secreted plant proteins and complexity in the plant cell wall proteome	Rose and Lee, 2010
Trafficking to the cell wall	Protein trafficking to the cell wall occurs through mechanisms distinguishable from default sorting in tobacco.	De Caroli., *et al.*, 2011
CBI, CESA, MTs	Cellulose biosynthesis inhibitors (CBIs) influence the trafficking and dynamics of CESAs or MTs	Brabham and Debolt, 2012; McFarlane *et al.*, 2014
TGN cell wall trafficking	Trans-Golgi network: an intersection of trafficking cell wall components.	Worden *et al.*, 2012
TGN ECHIDNA/Ypt cellwall	Trans-Golgi network localized ECHIDNA/Ypt interacting protein complex is required for the secretion of cell wall polysaccharides in Arabidopsis.	Gendre *et al.*, 2013
Texture, Turgor, cell wall	Cellular turgor contribute to fruit firmness and textural properties which is maintained by the water homeostasis within fruit and its distribution within the cell and in the cell wall	Seymour *et al.*, 2013
Tomato Cell wall, Pectin and softening	Cell wall composition of tomato fruit changes during development and inhibition of vesicle trafficking is associated with reduced pectin levels and reduced softening	Lunn *et al*., 2013
PME-HG	Computational approaches were proven to be very useful in assessing the functional dynamics of PME-HG complexes	Mercadante *et al.*, 2013, 2014
Cell wall of echidna mutants	Cell wall polysaccharides are mislocalized to the vacuole in echidna mutants.	McFarlane *et al.*, 2013
Strawberry FaRGLyase1	The strawberry (Fragaria x ananassa) fruit-specific rhamnogalacturonate lyase 1 (FaRGLyase1) gene encodes an enzyme involved in the degradation of cell-wall middle lamellae.	Molina-Hidalgo *et al.*, 2013
Expansin family	Genome-wide analysis of the expansin gene superfamily reveals grapevine-specific structural and functional characteristics	Dal Santo *et al.*, 2013
Cuticles	formation and function of plant cuticles	Yeats and Rose, 2013
Pectic arabinans inflorescence	Cell wall pectic arabinans influence the mechanical properties of *Arabidopsis thaliana* inflorescence stems	Verhertbruggen *et al.*, 2013
Cellulose	Cell biology of cellulose synthesis	McFarlane *et al.*, 2014
Cellwall	Cell wall, cytoskeleton, and cell expansion in higher plants	Bashline *et al.*,2014
Papaya fruit ripening	Analysis of papaya cell wall-related genes during fruit ripening indicates a central role of polygalacturonases during pulp softening.	Fabi *et al*.,2014
Cell wall green algae	The cortical cytoskeletal network and cell-wall dynamics in the unicellular charophycean green alga *Penium margaritaceum*	Ochs *et al.*,2014
Pectin disassembly	Fruit softening and pectin disassembly: an overview of nanostructural pectin modifications assessed by atomic force microscopy	Paniagua, *et al.*, 2014
Plasmodemata and cell wall	Roles and regulation of plant cell walls surrounding plasmodesmata	Knox and Benitez-Alfonso, 2014
CESA, CSC	CESAs are assembled into CSCs in either the endoplasmic reticulum (ER) or the Golgi apparatus and trafficked by vesicles to the PM	Bashline *et al.*,2014; McFarlane *et al.*,2014
Trafficking to	The Plant Secretory Pathway: an essential factory for building the	Kim and

cell wall	plant cell wall and for the trafficking of cell wall polysaccharides and glycoproteins	Brandizzi, 2014, 2016
CSC	Cellulase in Cellulose Synthase	Ueda, 2014
RAB Secondary Cell Wall	Emerging roles of small GTPases in secondary cell wall development	Oda and Fukuda, 2014
Mealiness apple PME	Multiscale investigation of mealiness in apple: an atypical role for a pectin methylesterase during fruit maturation.	Segonne *et al.*, 2014
Cellulose synthase	Computational and genetic evidence that different structural conformations of a non-catalytic region affect the function of plant cellulose synthase.	Slabaugh *et al.*, 2014
Cellulose synthase family	Evolutionary dynamics of the cellulose synthase gene superfamily in grasses.	Schwerdt *et al.*, 2015
PCWs	Do plant cell walls have a code	Tavares *et al.*, 2015
PMEs-PMEI	changes in pH and ionic strength influence the activity of PMEs, either directly or through the modulation of PME-PMEI interactions	Sénéchal *et al.*, 2015
Cutin	Plant cutin genesis: unanswered questions.	Domínguez *et al.*, 2015
XTHs, Expansin	Distribution of XTH, expansin, and secondary wall related CesA in floral and fruit abscission zones during fruit development in tomato	Tsuchiya *et al.*, 2015
MT and cellulose	Microtubules and cellulose biosynthesis: the emergence of new players.	Li *et al.*,2015
Pectoplate	PECTOPLATE: the simultaneous phenotyping of pectin methylesterases, pectinases and oligogalacturonides in plants during biotic stresses.	Lionetti, 2015
Cell wall and RabA	Impact of altered cell wall composition on saccharification efficiency in stem tissue of Arabidopsis RABA GTPase-deficient knockout mutants.	Lunn *et al.*,2015
Cell wall pectin	Modifications of cell wall pectin in tomato cell suspension in response to cadmium and zinc	Muschitz *et al.*, 2015
Cortical MTs	Cortical microtubule rearrangements and cell wall patterning	Oda, 2015
Apple cell wall and fruit softening	Lower cell wall pectin solubilisation and galactose loss during early fruit development in apple cv. 'Scifresh' are associated with slower softening rate.	Ng *et al.*,2015
Aarabinosyltransferases	A cascade of arabinosyltransferases controls shoot meristem size in tomato	Xu *et al.*,2015
Cytoskeleton and trichomes	Patterning mechanisms of cytoskeletal and cell wall systems during leaf trichome morphogenesis.	Yanagisawa *et al.*,2015
Xyloglucan guard cells	Functional analysis of cellulose and xyloglucan in the walls of stomatal guard cells of *Arabidopsis thaliana*	Rui and Anderson, 2015
Fiber1 kinesin MT trafficking	The fragile Fiber1 kinesin contributes to cortical microtubule-mediated trafficking of cell wall components	Zhu *et al.*,2015
Glycan and xyloglucan	structural insights into the enzymology of the ubiquitous plant cell wall glycan xyloglucan.	Attia and Brumer, 2016
β-galactosidase 4	Structural and functional analysis of tomato β-galactosidase 4: insight into the substrate specificity of the fruit softening-related enzyme.	Eda *et al.*,2016
Pectin, Hemicellulose in Grapes	Pectins, hemicelluloses and celluloses show specific dynamics in the internal and external surfaces of grape berry skin during ripening.	Fasoli *et al.*,2016
Cuticle	Cuticle structure in relation to chemical composition: re-assessing the prevailing model.	Fernández *et al.*,2016
Xylan Genes Sm and Pp	Evolutionary conservation of xylan biosynthetic genes in *Selaginella moellendorffii* and P*hyscomitrella patens*	Haghighat *et al.*,2016
fucosyltransferase1	Another step closer to understanding plant cell wall biosynthesis- the crystal structure of FUCOSYLTRANSFERASE1	Hofmann, 2016
Cell wall dynamics	The plant cell wall: a complex and dynamic structure as revealed by the responses of genes under stress conditions	Houston *et al.*, 2016

CSC	Cellulose synthase complexes act in a concerted fashion to synthesize highly aggregated cellulose in secondary cell walls of plants.	Li *et al.*,2016c
Expansin in wheat	Genomic location and expression analysis of Expansin gene family reveals the evolutionary and functional significance in *Triticum aestivum.*	Li *et al.*,2016e
CSC	cellulose synthase complex (CSC) in Arabidopsis are S-acylated	Kumar *et al.*,2016
Pectin, PGs	Pectins, Endopolygalacturonases, and Bioenergy	Latarullo *et al.*,2016
CWI and fruit set	Cell wall invertase promotes fruit set under heat stress by suppressing ROS-independent cell death	Liu *et al.*,2016f
SlExp1	Induced mutations in tomato SlExp1 alter cell wall metabolism and delay fruit softening.	Minoia *et al.*,2016
CSC	Comparative structural and computational analysis supports eighteen cellulose synthase in the plant cellulose synthesis complex	Nixon *et al.*,2016
Trafficking to cell wall	How do secretory products cross the plant cell wall to be released: A new hypothesis involving cyclic mechanical actions of the protoplast	Paiva, 2016
PCW-xyloglucan	Biosynthesis of the plant cell wall matrix polysaccharide xyloglucan	Pauly and Keegstra, 2016
GPAT6 and cutin	The Glycerol-3-Phosphate Acyltransferase GPAT6 from tomato plays a central role in fruit cutin biosynthesis.	Petit *et al.*,2016
Cutin	Ester cross-link profiling of the cutin polymer of wild-type and cutin synthase tomato mutants highlights different mechanisms of polymerization	Philippe *et al.*,2016
SYP123 and cell wall	The Root Hair Specific SYP123 regulates the localization of cell wall components and contributes to Rizhobacterial priming of induced systemic resistance	Rodriguez-Furlán *et al.*,2016
FUT1 and xyloglucan	Structure of *Arabidopsis thaliana* FUT1 reveals a variant of the GT-B class fold and provides insight into Xyloglucan Fucosylation	Rocha *et al.*,2016
Cellulose	Cellulose and callose synthesis and organization in focus,	Schneider *et al.*,2016
Xyloglucan cell wall seed geminration	Xyloglucan metabolism differentially impacts the cell wall characteristics of the endosperm and embryo during arabidopsis seed germination.	Sechet *et al.*,2016
Cutin tomato fruit	Cutinisation of tomato fruit epidermis: structural and morphological details	Segado *et al.*,2016
Cuticle tomato fruit	Ultrastructure of the epidermal cell wall and cuticle of tomato fruit during development.	Segado *et al.*,2016
Xylan and NMR	Folding of xylan onto cellulose fibrils in plant cell walls revealed by solid-state NMR.	Simmons *et al.*,2016
PME	Pectin Methylesterification impacts the relationship between photosynthesis and plant growth	Weraduwage *et al.*,2016
PCWs	Mapping nano-scale mechanical heterogeneity of primary PCW	Yakubov *et al.*,2016
CWS and morphogenesis	Regulation of meristem morphogenesis by cell wall synthases in Arabidopsis.	Yang *et al.*,2016b
Cell wall dynamics	Arabidopsis regenerating protoplast: a powerful model system for combining the proteomics of cell wall proteins and the visualization of cell wall dynamics	Yokoyama *et al.*,2016
IRX9, IRX10, and IRX14A	Asparagus IRX9, IRX10, and IRX14A Are Components of an active xylan backbone synthase complex that forms in the Golgi apparatus	Zeng *et al.*,2016
PMEI	Three Pectin Methylesterase inhibitors protect cell wall integrity for arabidopsis immunity to Botrytis	Lionetti *et al.*,2017
AtXTH3	The plant cell-wall enzyme AtXTH3 catalyses covalent cross-linking between cellulose and cello-oligosaccharide.	Shinohara *et al.*,2017
XD, GDSL	Control of secondary cell wall patterning involves xylan deacetylation by a GDSL esterase.	Zhang *et al.*,2017
PMEI	Combined Experimental and Computational Approaches Reveal Distinct pH Dependence of Pectin Methylesterase Inhibitors	Hocq *et al.*,2017
CESA	Functional Analysis of Cellulose Synthase (CESA) Protein	Kumar *et al.*,2017

SlEXP1	Overexpression of the carbohydrate binding module from *Sl-EXP1* modifies tomato fruit firmness and *Botrytis cinerea* susceptibility.	Perini *et al.*,2017a
Pectin	Pectic Oligosaccharide from tomato exhibiting anticancer potential on a gastric cancer cell line: structure-function relationship	Kapoor *et al.*, 2017
QTLs and cuticle	Uncovering quantitative trait loci and candidate genes for tomato fruit cuticular lipid composition using the *Solanum pennellii* introgression line population	Fernandez-Moreno *et al.*,2017
Expansin and texture	Measurement of expansin activity and plant cell wall creep by using a commercial texture analyzer	Perini *et al.*,2017b
AGT	Arabinogalactan glycosyltransferases target to a unique subcellular compartment that may function in unconventional secretion in plants.	Poulsen *et al.*, 2017
Trafficking to cell wall	The plant secretory pathway seen through the lens of the cell wall.	van de Meene *et al.*, 2017
PME Rice	Rice sucrose partitioning mediated by a putative pectin methyltransferase and homogalacturonan methylesterification	Xu *et al.*, 2017

AGT: Arabinogalactan glycosyltransferases, CBI: Cellulose biosynthesis inhibitors, CESA: Cellulose Synthase , CSC: Cellulose Synthase complex, EXP: Expansin, FUT: Fucosyltransferase, GPAT: Glycerol-3-Phosphate Acyltransferase, MT: Microtubules, PCW: Plant Cell Wall, PME: Pectin methyltransferase, PG: Polygalacturonases, QTL: Quantitative trait loci , RGLyase: Rhamnogalacturonate lyase, IL: Inbred Lines

1.5 Membrane trafficking and G-Proteins

There are large arrays of the GTP-binding proteins, which usually are referred to as G proteins. They are an important part of cell signalling. The G proteins are classified as heteromeric G proteins and monomeric G proteins. The heterotrimeric G proteins are famous as the G-protein-coupled receptors (GPCRs) involved in many signalling pathways in cells. These G proteins are made up from 16 Gα, five Gβ and 11 Gγ genes (McCudden *et al*., 2005). There are around 167 members of small mononmeric G proteins.

1.5.1 Ras super-family of GTPases

The Ras protein derives its name from the Rat sarcoma (Ras) a highly researched protein in oncology. The Ras superfamily constitutes of Ras, Rab (Ras like in Brain), Ral (Ras like), Rheb, Rap, Rac, Rho (Ras homolog), Arf (ADP ribosylation factor), Arl (ARF like), Sar, Ran (Ras like nuclear GTPases) (Rojas *et al*., 2012; Wennerberg *et al*., 2005; Pereira-Leal and Seabra, 2001; Pfeffer, 2017). The different sub families of Ras GTPases are listed in the table 1.6.

Rap GTPases are involved in the cellular adhesion. The Rab GTPase is the largest sub family of Ras GTPases. Rab GTPases are involved in the membrane trafficking and acts as director of vesicle trafficking. Rho GTPases are involved in the actin organization and cell cycle progression. Rho GTPases have been implicated in the

regulation of cell polarity, cell movement, cell shape and cell-cell and cell-matrix interactions, as well as in regulation of endocytosis and exocytosis (Ridley, 2001).

Table 1.6 Members of Ras Superfmaily of Small GTPases in Humans.

Family	Sub family	Members
Ras	Ras	H-Ras, K-Ras, N-Ras , M-Ras, R-Ras , R-Ras2
	Ral	Ral A, Ral B
	RheB	Rhe B, RheBL1
	Rap	Rap1A, Rap1B, Rap2A, Rap2B, Ras-3, Di-Ras1, Di-Ras2
	Rho	Rho A, Rho B, Rho C, Rho D, Rho E
	Rac	Rac1, Rac2, Rac3, Rop4, Rop5, Rop7, Rop9
Rab	Rab	Rab1,2,3,4,5,6,7,8,9,11,12,14,18,21,22,23,25,26,27,28,30,31,33,35,43
Arf	Arf	Arf1,2,4,5,6,8,
	Arl	Arl2,3,5,6,8,10,13
	Sar	Sar1
Ran	Ran	Ran, Ran E

The Arf family is involved in the vesicular transport by recruiting the coat protein complexes (Memon, 2004). Ran GTPases are involved in the nucleo-cytoplasmic transport of RNA and proteins (Weis, 2003). Ras, Rho, Rab, Ran and Arf all these small GTPases share a common mechanism and behave as binary switches. The human Ras superfamily contains 167 proteins containing classical families (39 Ras proteins, 30 Arfs, 22 Rhos and 63 Rabs. Schwartz *et al*. (2007) divided the Rab GTPase family into eight main functional groups (Pereira-Leal and Seabra, 2001) whereas, Stenmark (2009) classified it into nine groups. Recently, Klopper *et al*. in 2012 divided the family into six large groups using comparative genomics.

Ras is a small (21 kDa) GTPase that plays a central role in signal transduction in many signalling pathways. The Ras GTPases are activated by the conversion of GDP (guanosine diphosphate) into GTP (guanosine triphosphate) by the binary switch which is controlled by a number of guanine nucleotide exchange factors (GEFs), Ras guanine nucleotide release-inducing factors (GRFs) and Ras guanine nucleotide releasing proteins (GRPs) (Pereira-Leal and Seabra, 2001). There are number of Ras GTPase activating proteins (Ras GAPs) that brings about the conversion by hydrolyzing GTP to GDP (Barr and Lambright, 2010; Mishra and Lambright, 2016; Pandey, 2016).

1.5.2 Rab GTPases

The Rab family of proteins is a member of the Ras superfamily of monomeric G proteins called small GTPases. Rab GTPases (Ras-related in brain) are the key players in vesicle trafficking (Touchot *et al*., 1987). Rab GTPases have been found in many organisms including *Saccharomyces cervisiae* (11 members), *Cenorhabtiditis elegans* (29 members), *Drosophila melanogaster* (26 members) (Bock *et a*l., 2001), *Arabidopsis thaliana* (57 members), Recently Wang *et al*., (2014a) identified a total of 52 Rab genes from catfish and Li and Guo (2017) reported 87 Rab GTPases in Cotton (*Gossypium raimondii*).

The localizations and vesicle transport pathways of different Rab GTPases in an animal cell is different as compared to plants. Different Rab GTPases are listed with their localization and function in the membrane trafficking pathways in Table 1.7. RAB1/rab1p is located at endoplasmic reticulum (ER) exit sites and the pre-Golgi intermediate compartment (IC) which mediates ER–Golgi trafficking. RAB2 is located at the IC might also regulate Golgi–ER trafficking. The Golgi-localized RAB6, RAB33 and RAB40 mediate intra-Golgi trafficking. RAB33 together with RAB24 regulates the formation of autophagosomes. RAB8 mediates constitutive biosynthetic trafficking from the *trans*-Golgi network (TGN) to the plasma membrane and also participates in GLUT4 vesicle translocation (with RAB10 and RAB14) and ciliogenesis (with RAB17 and RAB23) (Ishikura *et al*., 2008 ; Sadacca, *et al*., 2013).

RAB3, 26, 27 and RAB37 mediate various types of regulated exocytic events and RAB27 also mediates the translocation of melanosomes to the cell periphery. RAB32 and RAB38 are involved in the biogenesis of melanosomes and RAB32 also controls mitochondrial fission. RAB13 regulates the assembly of tight junctions between epithelial cells. RAB18 controls the formation of lipid droplets. RAB22 mediates trafficking between the TGN and early endosomes and *vice versa*. RAB5, which is localized to early endosomes, phagosomes, caveosomes and the plasma membrane, mediates endocytosis and endosome fusion of clathrin-coated vesicles (CCVs), macropinocytosis (with RAB34) and maturation of early phagosomes (with RAB14 and RAB22). RAB21 mediates integrin endocytosis.

RAB11 and RAB35 mediate slow endocytic recycling through recycling endosomes, whereas RAB4 mediates fast endocytic recycling directly from early endosomes. RAB15 is involved in the trafficking from early endosomes to recycling endosomes and in the trafficking from apical recycling endosomes to the basolateral plasma membrane. RAB17 and RAB25 control trafficking through the apical recycling endosomes to the apical plasma membrane. The late endosome-associated RAB7 mediates maturation of late endosomes and phagosomes, and their fusion with lysosomes. Another late endosomal GTPase, RAB9, mediates trafficking from late endosomes to the TGN (Stenmark, 2009). The difference in the number of *Rab* genes are mainly caused by gene duplications, leading to the presence of paralogs (*e.g*. Rab5a, Rab5b and Rab5c) (Table 2). Orthologous Rab genes from various species, as well as their paralogs, are structurally highly conserved (Eoin *et al*., 2012; Touchot *et al*., 1987). In large yellow croaker, Rab8 was up-regulated after infection with *Vibrio parahaemolyticus* (Han *et al*., 2011). Cytokinesis bridge instability leads to binucleated cells that can promote tumorigenesis *in vivo* (Fujiwara *et al*., 2005). Membrane trafficking is crucial for animal cell cytokinesis (Glotzer, 2001) and several endocytic pathways regulated by distinct GTPases (Rab11, Rab21, Rab35, ARF6, RalA/B) contribute to the post-furrowing steps of cytokinesis (Wilson *et al*., 2005).

Table 1.7 Rab GTPases, their intracellular localization and known pathways.

Rab	Localization	Function in Membrane Traffic Pathway	References
RAB1 A,B	ER, Golgi	ER to Golgi, intra-Golgi	Plunter,1991 Sannerud *et al*., 2006
RAB2 A,B	ER, ER-Golgi intermediate compartment,	ER to Golgi, maturation of pre-Golgi intermediates, Golgi-ER transport	Tisdael *et al* ., 1996 Buffa *et al* ., 2008
RAB3 A,B,C,D	Secretory vesicles, plasma Membrane	Regulated exocytosis Synaptic vesicles, secretory granules neurotransmitter release	Brondyk et al., 1995 Aligianis *et al*., 2005
RAB4 A,B,C	Early endosome, recycling endosome	Endocytic recycling	Bielli, 2001
RAB5 A,B,C	Early endosome, PM, CCVs,	Early endosome fusion, phagosome maturation, growth factor signalling Caveosomes,	Bucci *et al*., 1992
RAB6 A,B,C	Golgi	Endosome to Golgi, intra-Golgi transport, Golgi to ER	Barr,1999 Del Nery, 2006
RAB7 A,B	Late endosomes, lysosomes vacuole, melanosomes,	Late endosome to lysosome EE-LE trafficking, lysosome biogenesis, phagosome maturation	Bucci *et al*., 2000 De Lucca 2008
RAB8 A, B	Cell membrane, vesicles, primary cilia TGN	Exocytosis, TGN/RE to plasma membrane TGN-PM trafficking, ciliogenesis, basolateral and dendritic sorting, adherens junction assembly	Ang *et al*., 2003 Chen *et al*., 2001

RAB9 A, B	Late endosomes, Golgi	Endosome to TGN, LE-TGN trafficking	Diaz *et al*., 1997
RAB10	Golgi, endosomes,	Exocytosis, TGN/RE to plasma membrane basolateral sorting	Babbey *et al*., 2006
RAB11 A,B	Golgi, early endosomes recycling endosome	TGN/RE to plasma membrane Endocytic recycling, cytokinesis	Lindsay *et al*., 2002
RAB12	Golgi, secretory vesicles TGN, SGs	Exocytosis Transport from cell periphery to pericentriolar region	Iida *et al*., 1996
RAB13	TGN, recycling endosome,	TGN/RE to plasma membrane Biogenesis of tight junctions	Yamamura *et al*., 2008
RAB14	Golgi, early endosome,	TGN/RE to plasma membrane; apical membrane targeting Phagosome maturation, TGN-EE trafficking	Junutula *et al*., 2004
RAB15	Early/sorting endosome, recycling endosome	Sorting endosome/RE to plasma membrane Trafficking through EEs, endocytic recycling	Zuk *et al*., 1999
RAB17	recycling endosome	Transcytosis, Trafficking through apical REs,	Lütcke *et al*., 1993
RAB18	Golgi, lipid droplets ER, Lipid droplets, SGs	ER-Golgi trafficking, Lipid droplet Formation of lipid droplets from ER, negative regulator of neuroendocrine secretion	Dejgaard *et al*., 2008
RAB19	Golgi	Unknown	Sinka *et al*., 2008
RAB20	Golgi, endosome	Apical membrane recycling , Connexin-43 trafficking	Amillet *et al*., 2006
RAB21	Early endosome	Endosomal transport Integrin endocytosis, cytokinesis	Pellinen *et al*., 2006
RAB22	Early endosome, TGN	Endosomal transport, protein recycling to plasma membrane EE-TGN and TGN-EE transport, attenuation of phagosome maturation	Kauppi *et al*., 2002
RAB23	PM, endosome PM, EEs	Protein recycling/transport to plasma Membrane Phagosome-lysosome fusion,	Olkkonen *et al*., 1994
RAB24	ER	Autophagosome formation Induction of autophagy	Munafó and Colombo, 2002
RAB25	Recycling endosome	RE (apical) to plasma membrane Transport through apical REs, integrin recycling	Cheng *et al*., 2006 Caswell *et al*., 2007
RAB26	Secretory granules	Exocytosis Regulated exocytosis of SGs	Barr, 1999
RAB27 A, B	Melanosomes SGs, T-cell granules	Exocytosis Regulated secretion, Myosin Va recruitment to melanosomes and T-cell granules	Bahadoran *et al*., 2001
RAB30	ER, Golgi	Unknown	Thomas *et al*., 2009
RAB31	TGN, endosome	TGN-PM and TGN-EE trafficking	Chen *et al*., 1996a,b Bao *et al*., 2002
RAB32	Melanosomes, mitochondria	TGN to melanosome, mitochondrial fission & dynamics	Masuda *et al*., 2000
RAB33 A,B	Golgi, dense-core vesicles	Golgi-ER trafficking, Autophagosome formation	Itoh *et al*., 2008
RAB34	Golgi, macropinosomes macropinosomes,	Intra-Golgi transport, peri-Golgi positioning of lysosome Macro-pinocytosis, lysosome positioning	Chen *et al*., 2010
RAB35	PM, endosome	RE to plasma membrane, actin assembly Endocytic recycling, cytokinesis, phagosome-lysosome fusion, immunological synapse formation	Kouranti *et al*., 2006
RAB36	Golgi	Unknown	Itoh *et al*., 2008
RAB37	Secretory granule	Exocytosis, Mast cell degranulation	Masuda *et al*., 2000

RAB38	Melanosomes	TGN to melanosome, Melanosome biogenesis	Loftus *et al*., 2002
RAB39	Golgi	Unknown	Becker *et al*., 2009 Chen *et al*., 2010
RAB40 A,B,C	Golgi, Recycling endosome	Endosome/intracellular transport	Lee *et al*., 2007
RAB41	Golgi	Unknown	Fukuda *et al*., 2008
RAB43	ER, Golgi Golgi	ER-Golgi trafficking	Dejgaard *et al*., 2008
RAB45	Peri-nuclear region	Unknown	Shintani *et al*., 2009

Abbreviations: PM- plasma membrane; ER-endoplasmic reticulum; EE- early endosome, LE- late endosome; RE- recycling endosome; IC- pre-Golgi intermediate compartment; TGN, trans-Golgi network, SG- Secretory granule.

1.6 Intracellular membrane traffic

Rab GTPases regulate many steps of membrane traffic, including vesicle formation, vesicle movement and membrane fusion. These processes make up the route through which cell surface proteins are trafficked from the Golgi to the plasma membrane and are recycled back. Rab GTPases are involved in processes in intracellular membrane traffic.

1.6.1 Mechanistic insight into the intracellular membrane traffic:

Membranes are the physical boundaries of the cell and cellular organelles. The cell membrane represents a permeability barrier between the internal and the external aqueous mileux. The hydrophobicity contributes not only to the cohesive force that stabilizes the phospholipid bilayer but also holds the integral proteins in place.

The endoplasmic reticulum (ER) reaches out from the nuclear envelope to the cortical regions of the cell and may be subdivided into several functionally distinct domains (Staehelin, 1997). The ER is the entry point into the endomembrane system for newly synthesized proteins. Proteins and lipids synthesized in endoplasmic reticulum provide a foundation for the assembly of all compartments comprising the exocytic and endocytic pathways. The vesicle cargo moves from ER through exocytic and endocytic compartments carried by lipid bound transport containers that include both small vesicles (60-80 nm in diameter) and elongated membrane tubules. The Golgi apparatus is a major sorting station, delivering cargo proteins to multiple destinations. Plant cells have between several and hundreds of Golgi stacks (Staehelin & Moore, 1995). The complexity of the trafficking problem is illustrated by the fact that this process simultaneously moves thousands of different proteins efficiently and precisely between different

compartments without mix-up. The intracellular transport must be coordinated with constantly changing needs of the cells and tissues in the response of the environmental and cellular physiology. The intracellular vesicular trafficking takes place in three steps. The first step is selection of the cargo wherein the so called adaptor proteins couple sorting and signals on specific cargo molecules to the transport machinery. The second step involves the formation of the container or vesicle.

The protein form coat lattices that promote the pinching off of a part of the donor compartment to form the vesicle. A variety of coat lattice facilitates vesicle formation from different exocytic and endocytic compartments. The last step is targeting and fusion of the vesicle to the next compartments/ organelle membrane. Membrane associated tethering and fusion protein direct the delivery of the transport containers to the right targeted compartment.

During the course of evolution several large gene families have evolved to route thousands of different proteins to their proper cellular locations. Small guanosine triphosphatase (GTPase) regulate the three steps by controlling the assembly and the disassembly of transport vesicle. In the case of vesicular membrane trafficking, the cycle of the activation through the exchange of GDP for GTP and inactivation is by GTP hydrolysis.

The mechanism of forward transport *e.g.* from ER to Golgi along the secretory pathway in an anterograde direction begins with the movement of vesicle from ER to Golgi. COP II mediates cargo selection and assembly of targeting and formation of the compartment and then is recycled back, whereas, COP-I vesicles facilitate the retrograde transport pathways (Popoff *et al.,* 2011). In case of yeast 40-50 gene products are required to complete the budding and fusion of vesicle in anterograde and retrograde pathway between ER and Golgi.

Genetic studies in yeast as well as biochemical methods such as cell fractionation assays have contributed to the current understanding of membrane trafficking. Cell fractionation assays detect transfer of cargo between membrane compartments prepared from cell homogenates by utilizing the biochemical properties of organelles.

1.7 General features of the vesicular transport

Cargo selection, vesicle budding and fusion all occur with accuracy and fidelity. Membrane integrity is maintained at all steps. Along with this the topology, orientation of the lipid and protein in the membrane bilayer is maintained by vesicular carriers. One side always faces the cytoplasm and the other to the lumen of ER.

Step 1: Cargo selection through sorting motifs

Every vesicle that buds from a particular membrane compartment incorporate proteins or polysacchharides destined for transport. Molecular machinery which generates vesicle must recognize recruitment signals, a short peptide sequence, is on the transported cargo proteins. Fidelity factor recycle between ER and golgi generates a threshold for being recognised efficiently by export machinery. Presence of fidelity factor and adaptor protein suggest both the exocytic and endocytic pathways may consist of a network of signalling cascades that can adjust dynamically to the changing needs of cell.

Step 2: COP II vesicular coat assembly and budding from ER

Packaging of cargo into transport vesicle involves the coat complex II (COP II). The small GTPase Sar1 regulates the assembly of COP II vesicular coat. *Arabidopsis* encodes three SAR1 GTPases and two SEC12 proteins of which each one was isolated by complementation of yeast mutants and shown to associate with the ER (d'Enfert *et al*. 1992; Bar-Peled & Raikhel, 1997; Vernoud *et al*. 2003). Assembly of COP II coat begins where ER membrane associated GEF; Sec12 activates Sar1 by exchange of GDP to GTP. A hydrophobic motif at the N terminus of Sar1 recognises Sec12. The activation of Sar1 GTP coordinates recruitment of molecules (through sorting motifs). The function of Sec 23/24 is to stabilize the activated Sar1, which then helps in the assembly of the cargo and other membrane compartment into larger assembly which acts as a pre-budding complex (Barlowe, 2003). The formation of this complex is first committed step in the assembly of the coat. Next another cytosolic protein sorting complex is recruited. This mediates further coat assembly, by linking the cargo container pre-budding complex into a more extensive molecular lattice; a process that drives membrane invagination in

order to form a bud on the surface of ER. After fusion the COP-II vesicle is released to cytoplasm.

The life cycle of a transport vesicle (fig1.2) starts from its budding, transport, docking to the target membrane followed by fusion and then disassembly. The process of budding of a vesicle is initiated by recruiting a small Arf/Sar GTPase on the donor membrane by its guanine nucleotide exchange factor (GEF), which then is followed by the recruitment of coatamers or coat components which are bound to the donor membrane wherein the cargo proteins diffuse into the budding site where they interact with the cargo receptors. The membrane forms a coated bud which results into the formation of a vesicle. Then the GTPase activity is stimulated by a GTPase activating protein (GAP) resulting in the uncoating and release of the GTPase and the coat proteins into the cytosol. Thus the vesicles are transported from their budding site from donor membrane to the acceptor compartment through the cyto-skeletal network including actin, and the molecular motors like myosin, dyneins and kinesin. Now the GTP-bound Rab protein directs the vesicle to the targeted sites of fusion with the help of elongated coiled-coil tethering factors.

After the docking step, an R-SNARE (VAMP) assembles with a Qa-SNARE (syntaxin), a Qb-SNARE (SNAP N) and a Qc-SNARE (SNAP C) to form a parallel, four-helical bundle which forms the core fusion complex. The zipper like mechanism of these parallel helices brings the vesicle and acceptor membrane close together initiating the fusion of the vesicle and acceptor membrane (Bock *et al*., 2001). Now the GDP-dissociation inhibitor (GDI) releases the Rab-GDP from the membrane, which is then followed by the disassembly of the SNARE complex by the ATPase N-ethylmalemide-sensitive factor (NSF) and a-SNAP so that SNAREs can be recycled (Bock *et al*., 2001, Sanderfoot *et al*., 2007).

Step 3: Vesicular Delivery

Targeting of the vesicle is daunting task given the myriad of exocytic and endocytic compartment in the cell. A targeting complex has a defined destination, identity of which is conferred by the docking complex which recognizes the incoming vesicle.

The Rab GTPase regulates the assembly of the delivery system. They function as molecular switches to control the protein-protein interaction directing

the formation of the targeting and docking complexes. *Rab* genes are conserved from yeast to humans indicating Rabs control ancient biochemical process. They are post translationally modified by the two geranylgeranyl lipids at conserved residues found at their carboxyl termini. This modification is essential for function and it facilitates Rab association with the membrane bilayer. Cysteines are included in a segment of 30 amino acids that vary between Rabs, which direct them to sub-cellular location. Targeting and docking complexes assembled by different Rab GTPase generate molecular zip codes that specify traffic between various compartments.

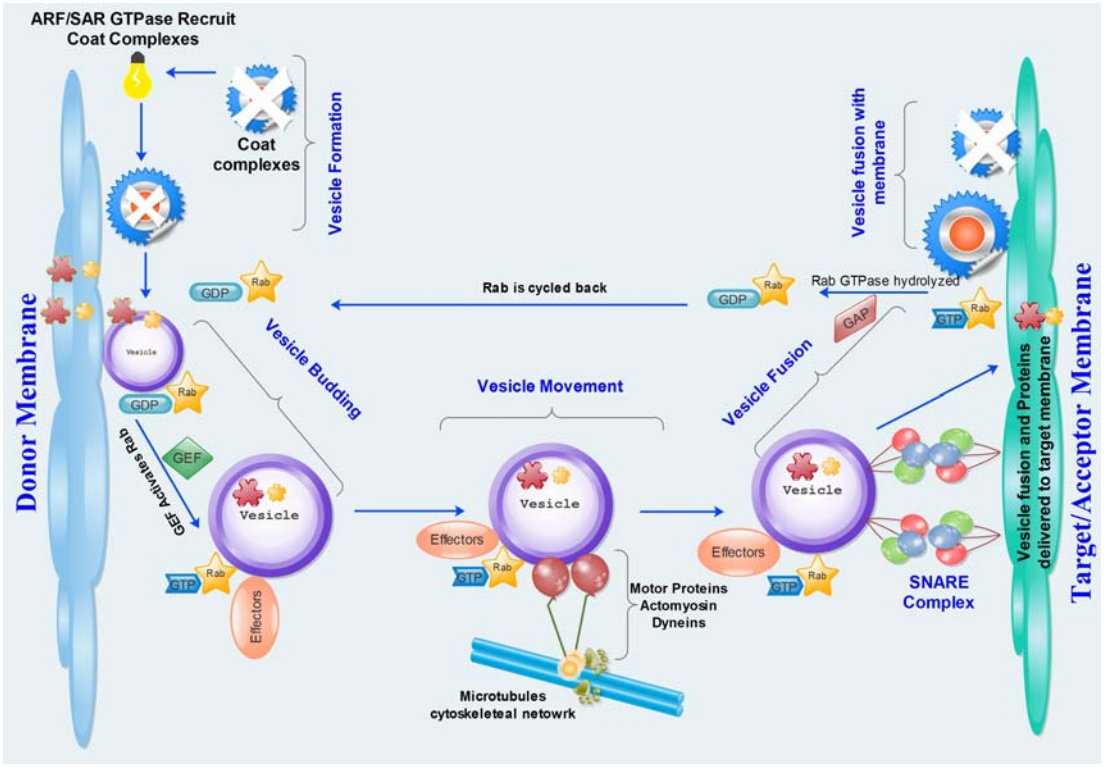


Fig. 1.2 Vesicle Transport Cycle

The schematic diagram of vesicle transport from donor to acceptor membrane is shown. Vesicle formation, budding, transport and fusion are highlighted. The recruitment of coat complexes by ARF GTPase at donor membrane, the activation of the Rab GTPase and the movement of Vesicle on the cytoskeletal structures is carried through the microtubules with the help of effectors and molecular motors. The fusion with the target membrane with the help of SNARE complexes takes place and the Rab GTPase is hydrolyzed by GAP and is cycled back (The figure was drawn using freely available drawing software EDraw).

1.8 Rab GTPase Cycle

The Rab GTPases switches between two conformations, an inactive form bound to GDP (guanosine diphosphate), and an active form bound to GTP (guanosine triphosphate). Conversion of the GDP-bound Rab into the GTP-bound form occurs through the exchange of GDP for GTP, which is catalysed by a guanine nucleotide exchange factor (GEF) and causes a conformational change.

As shown in fig.1.3 the GTP-bound 'active' conformation is recognized by multiple effector proteins and is converted back to the GDP-bound 'inactive' form through hydrolysis of GTP, which is stimulated by a GTPase-activating protein (GAP) and releases an inorganic phosphate (Pi).

The newly synthesized Rab, in the GDP-bound form, is recognized by a Rab escort protein (REP). This results in the formation of the RAB GDP-Rab GEF complex. There are different REP for specific Rab GTPases. The REP presents the Rab GDP to a geranylgeranyl transferase (GGTase), which geranylgeranylates the Rab on one or two carboxy-terminal Cys residues.

The geranylgeranylated, GDP-bound Rab is recognized by Rab GDP dissociation inhibitor (GDI). Targeting of the Rab–GDI complex to specific membranes is mediated by interaction with a membrane-bound GDI displacement factor (GDF). GDI is then released by the GDI Displacement Factor (GDF). The free GDI is again cycled back for the next cycle to bind the next geranylated Rab.

The conversion of inactive Rab GDP to active Rab GTP is brought by the Rab Guanine Exchange Factor (GEF). Once activated Rab GTP interacts with the effectors and binds to the vesicles. In the different membrane trafficking pathways from ER to Golgi, Golgi to PM or Golgi to endosomes, vacuoles, Rab GTPase plays active role in correct trafficking and targeting of the vesicles. The Rab GTPases specifically binds and delivers the vesicle through the highly complex network of molecular motors like Actin, Myosin, dyneins, through the cytoskeletal structures of microtubules as shown in fig 1.2. The fusion is brought about by the SNAREs and active role of dynamin GTPase pinching at the target membrane of the organelles or plasma membrane.

Once the target membrane is recognized the vesicle is deliverd and the fusion takes place followed by delivery of the cargo to the target membrane. Once

the cycle is over Rab GTP is hydrolyzed by Rab GTPase Activator Protein (GAP) to Rab GDP by the release of inorganic phosphate (Pi). Thus after release of Pi, the Rab GDP is cycled back and ready for other cycle to bind REP. Fig.1.3 is schematic representation of the various stepwise process involved in the Rab GTPase cycle.

1.8.1 Different classes of Rab GTPases in plants

In *Arabidopsis*, the fifty seven Rab GTPases have close homology to only eight of the types found in mammals. The plant Rab family consists of eight subfamilies, designated AtRabA–AtRabH, each with counterparts in yeast or animals. These are further divide into 18 subgroups, most of which are plant-specific and of unknown function (Pereira-Leal and Seabra, 2001; Rutherford and Moore, 2002; Vernoud *et al*., 2003; Deneka *et al*., 2003). Few experimental data available to describe Rab GTPases are mainly restricted to the RabA, RabF and RabG subfamilies.

Most notably, the two mammalian Rab11 GTPases correspond to the *Arabidopsis* RabA clade, which has 26 members, divided into six sub groups (RabA1 – RabA6). The RabA2 subgroup is closely related to human Rab11 and Rab25, which are generally thought to be associated with the polarised recycling of proteins at the PM. This has led to the hypothesis that these sub groups may have evolved different functions (Rutherford and Moore, 2002). RabB (Rab2) and RabD (Rab1) are expected to be involved in ER to Golgi transport in plants (Batoko *et al*., 2000; Cheung *et al*., 2002). RabC (Rab18) and RabF (corresponding to mammalian Rab5 and/or Rab22), and RabG (Rab7) proteins are expected to be associated with endocytosis. RabE is related to the Golgi to PM associated Rabs of mammals such as Rab8 and seems to have the same role in plants (Zheng *et al*., 2005). RabFs may be involved in either endocytosis or vacuolar trafficking (Ueda *et al*., 2001; Grebe *et al*., 2003; Sohne *et al*., 2003; Bolte *et al*., 2004; Kotzer *et al*., 2004; Lee *et al*., 2004). RabG (Rab6) will complement its yeast homologue, *Ypt6*, which is implicated in retrograde Golgi to ER transport (Bednarek *et al*., 1994). Lycett (2008) summarised the old and new nomenclature and functions of Rab GTPase in plants, as shown in Table 1.8. Table 1.9 shows the recent studies on Small GTPases RAB (AtoH), and their regulators GAP, GEF, GDI and GGTase and other small GTPases RAN, ROP, ARF, ARL and SAR.

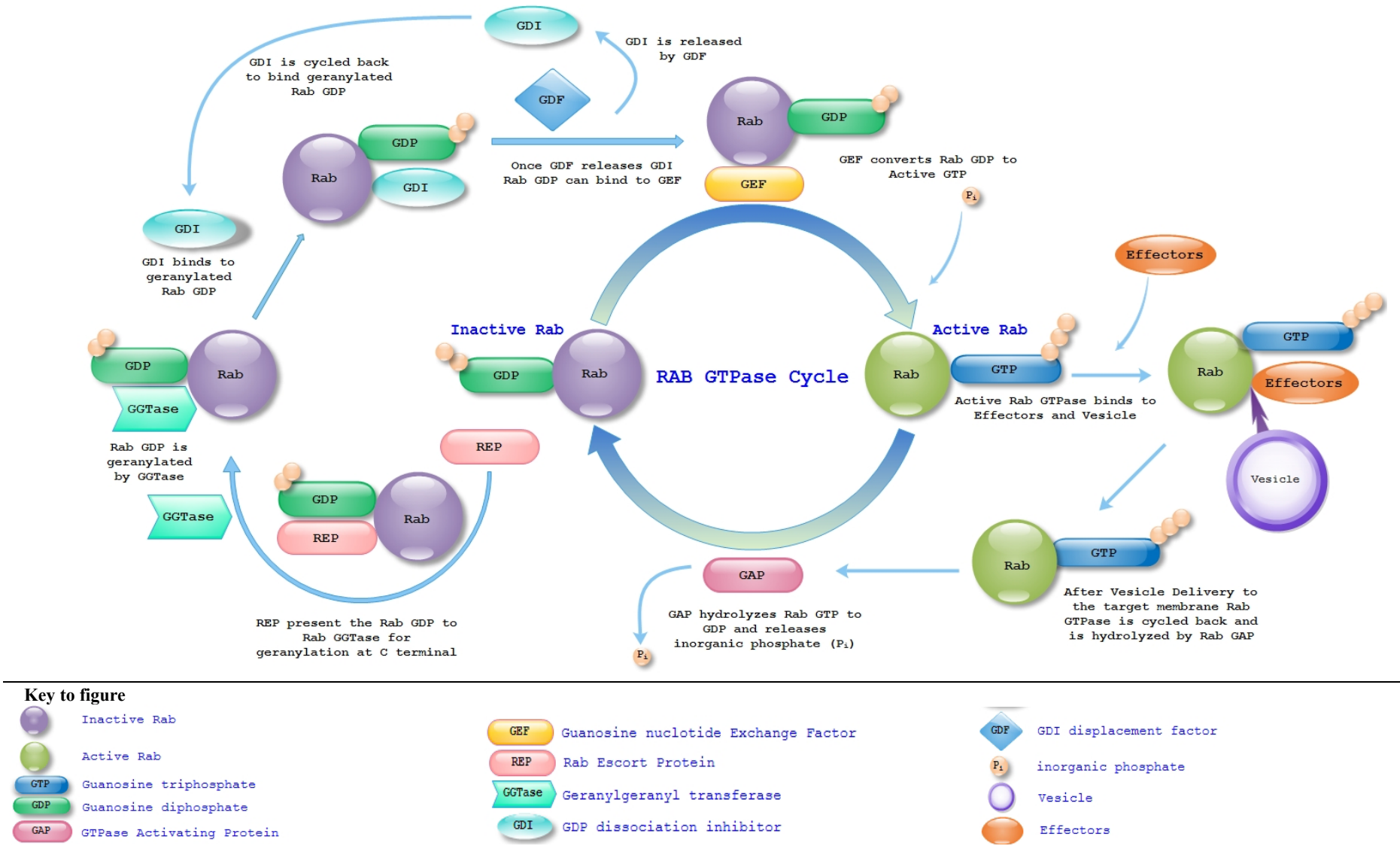


Fig. 1.3 Rab GTPase cycle

Rab GTPase cycles between to conformations. The newly synthesized Rab GDP interacts with the Rab Escort Protein (REP), which escorts the Rab GDP to Rab geranylgeranyl transferase (GGT). This complex is then recognised by the Rab GDP dissociation inhibitor (GDI) to bind donor membrane. GDI is then released by the GDI Displacement Factor (GDF). The conversion of inactive Rab GDP to active Rab GTP is brought by the Rab Guanine Exchange Factor (GEF). Once activated Rab GTP interacts with the effectors and delivers the vesicles/cargo to the target membrane. Once the cycle is over Rab GTP is hydrolyzed by Rab GTPase Activator Protein (GAP) to Rab GDP by the release of inorganic phosphate (Pi). (Fig. was designed using freely available diagram designer)

Table 1.8 Nomenclature and functions of different Rab GTPase in Plants (Compiled by Lycett, 2008)

New *Arabidopsis* names	Equivalent mammalian groups	Function based on mammalian studies	Probable function in plants
Rab A	Rab11	Recycling through endosomes and Golgi	Several subgroups in plants so functions possibly diverse. Associated with Golgi vesicles and TGN/endosomes. Golgi →PM trafficking Also involved in signalling
Rab B	Rab2	cis-Golgi. ER →Golgi trafficking	ER →Golgi trafficking
Rab C	Rab18	ER-Golgi trafficking	Lipid droplet Formation and trafficking
Rab D	Rab1	ER →Golgi trafficking	ER →Golgi trafficking
Rab E	Rab8/10/12	Constitutive exocytosis, especially in polarized cells. TGN → PM	Golgi →PM
Rab F	Rab5/22	Early exocytic pathway. Early endosome fusion	PM →endosome and/or PVC →vacuole. Osmotic stress
Rab G	Rab7	Late endocytic pathway Intraendosomal and endosome → lysosome transport	PM →vacuole EE-LE trafficking Osmotic stress
Rab H	Rab6	Retrograde intra-Golgi and Golgi → ER transport	Stress responses. PVC/late endosome →vacuole trafficking

ER: Endoplasmic reticulum; PM: Plasma membrane; PVC: Pre-vacuolar compartment., EE- Early Endosome, LE- Late Endosome

Table 1.9 Recent studies on Small GTPases RAB (A to H), and their regulators GAP, GEF, GDI and GGTase and other small GTPases RAN, ROP, ARF, ARL, SAR.

Genes	Remarks	References
RABA		
RabA	RabA is localized to TGN and is involved in cell plate formation and tip growth	Preuss *et al.*,2004; Chow *et al.*,2008; Szumlanski and Nielsen, 2009; Bottanelli *et al.*,2011; Kang *et al.*,2011; Feraru *et al.*,2012; Asaoka *et al.*,2013; Choi *et al.*,2013 Berson *et al.*,2014; Kirchhelle *et al.*,2016,
RabA2 and A3	Rab-A2 and Rab-A3 GTPases define a trans-Golgi endosomal membrane domain in Arabidopsis that contributes to the cell plate formation	Chow *et al.*,2008
RabA2	RabA2 RNAi knock out impairs root hair formation it growth as well as affects pre-infection events of symbiosis in common bean (*Phaseolus vulgaris*)	Blanco *et al*., 2009

VvRabA5e	Cellular localisation of VvRops and VvRabA5e, small GTPases developmentally regulated in grape berries.	Conéjéro *et al.*,2010
RabA	Null mutants of individual RABA genes impact the proportion of different cell wall components in stem tissue of *Arabidopsis*	Lunn *et al*., 2013
Rab-A1c	Role of Rab-A1c during cytokinesis in Arabidopsis	Qi *et al.*,2011b, 2013
RabA	Role of RabA in subcellular trafficking of the FLAGELLIN SENSING2 receptor	Choi *et al.*,2013
RabA1	Role of AtRabA1 in salinity stress tolerance	Asaoka *et al.*,2013
AtCPRabA5e	Arabidopsis 'chloroplast localized Rab GTPase' protein CPRabA5e is involved in stress, development, thylakoid biogenesis and vesicle transport.	Karim *et al.*,2014
RabA1d	RabA1d is localized at TGN and is involved in cell plate formation and oscillatory root hair growth	Berson *et al.*,2014
RabA4c and PMR4	Interaction of AtRabA4c with PMR4 effector results in complete penetration resistance to powdery mildew	Ellinger *et al.*,2014
RabA4b PUB13, PI4Kβ1/β2	Recruitment of PLANT U-BOX13 and the PI4Kβ1/β2 phosphatidylinositol-4 kinases by AtRabA4B plays important roles during salicylic acid-mediated plant defense signalling	Antignani *et al.*,2015
RabA	Impact of altered cell wall composition on saccharification efficiency in stem tissue of Arabidopsis RABA GTPase-deficient knockout mutants.	Lunn *et al.*,2015
RAB	Role of GTP-binding proteins as novel gene target for biofuel production.	Voshol *et al*.,2015
RabA5c	Specification of geometric edges by a plant Rab GTPase is essential for cell-patterning during Arabidopsis organogenesis.	Kirchhelle *et al.*,2016
RabA5c	Role of RabA5c in plant cell shape and trafficking to cell edges	Rahni *et al*., 2016
RabA2	RabA2 is required for progression and maintenance of membrane integrity of infection threads during root nodule symbiosis	Dalla Via *et al.,* 2017
Rab11, MyosinV Dynein and Kinesin	Transport of fungal RAB11 secretory vesicles involves myosin-5, dynein/dynactin/p25, and kinesin-1 and is independent of kinesin-3	Peñalva *et al.*,2017
RabB/Rab2		
RAB2A	RAB2A controls MT1-MMP endocytic and E-cadherin polarized Golgi trafficking in breast cancer	Kajiho *et al.*,2016
Rab2 and RabS	Rab2 and RabS colocalize with the Golgi and contractile vacuole system and regulate osmoregulation in *Dictyostelium discoideum*	Maringer *et al.*,2016
Rab2	Rab2 promotes autophagic and endocytic lysosomal degradation	Lőrincz *et al.*,2017
Rab18/RabC		
Rab18	Rab18 localizes to lipid droplets and ER derived membranes	Ozeki *et al.*,2005
Rab18	Rab18 facilitates dengue virus infection by targeting fatty acid synthase to sites of viral replication	Tang *et al.*,2014
Rab18 and Rab18 GEF	Rab18 and a Rab18 GEF complex are required for normal ER structure	Gerondopoulos *et al.*, 2014
Rab18	Bacterial-induced expression of RAB18 protein in salinity stress in Rice	Jha *et al.,* 2014
Rab18	Rab18 in watermelon (*Citrullus lanatus*) was highly expressed in root, stem and leaf, moderately expressed in flower but weakly expressed in the fruit	Xinli and Lei, 2015
RAB18 and MiR455-5p	MiR-455-5p down-regulates RAB18 and acts as a tumor suppressor in gastric cancer	Liu *et al.*,2016b
Rab18	RAB18 modulates macro-autophagy and proteostasis	Feldmann *et al.*,2017
TaRab18	Transgenic plants with over-expressed TaRab18 showed a strong hypersensitive response at the early infection stage of wheat stripe rust.	Jiang *et al.,* 2017
Rab1/RabD		
Rab-D1 and Rab-D2	Rab-D1 and Rab-D2 GTPases exhibit distinct but overlapping interactions in the early secretory pathway	Pinheiro *et al.*,2009
RabD, RabA	At TRAPPII, AtRabA and At RabD polar protein trafficking in	Qi *et al.*,2011a,2013

and TRAPPII	trans-Golgi network	
Rab1, COPI,II	Rab1b regulates COPI and COPII dynamics in mammalian cells	García *et al.*,2011
AtRabD2b AtRabD2c	AtRabD2b and AtRabD2c in pollen development and pollen tube growth	Peng *et al.*,2011
RabD	Manipulating Rab GTPase activity in wheat to improve gluten quality for breadmaking.	Tyler *et al.*,2015
RabD	RabD GTPase in wheat and its role in gluten quality	Tyler *et al.*,2015
Rab1	Role of Rab1 in the secretory pathway of *Leishmania*	Bahl et al.,2015
Rab1A	*Plasmodium falciparum* Rab1A localizes to Rhoptries in Schizonts	Morse *et al.*,2016
Rab1A and golgin-84	Rab1A mediates proinsulin to insulin conversion by maintaining Golgi stability through interactions with golgin-84 in β-cells	Liu *et al.*,2016e
Rab1	Role of Rab1 in cell signalling, cancer and other diseases	Yang *et al.*,2016c
Rab1b	Spatio-temporal Study of Rab1b dynamics and function at the ER-Golgi interface	Martinez et al.,2016
Rab1	Use of an ensemble of clustering methods in identification of Rab1 binding sites	Lukman *et al.*,2017
RabE/Rab8		
RabE and RabD	A Rab-E GTPase mutant acts downstream of the Rab-D subclass in biosynthetic membrane traffic to the plasma membrane in tobacco leaf epidermis	Zheng *et al.*,2005
RabE	Subcellular localization and functional analysis of AtRabE.	Speth *et al.*,2009
RabE	Rab-E GTPases exhibit a novel interaction with a plasma-membrane phosphatidylinositol-4-phosphate 5-kinase	Camacho *et al.*,2009
Rab8	Role of Rab8 in regulation of cell shape	Peranen *et al.*,2011
Rab8a	Rab8a regulates the exocyst-mediated kiss-and-run discharge of the contractile vacuole in *Dictyostelium*	Essid *et al.*,2012
RabE1	Characterization of NbRabE1 in *Nicotiana benthamiana*	Ahn *et al.*,2013
RabE1d13-185 GTPase	Expression, purification and G- nucleotide binding characterization of At RabE1d13-185 GTPase	Gao *et al.*,2016
Rab8	Evolution of Rab8 and the effector bMERB	Rai *et al.*,2016
Rab8a	Targeting of Rab8a by protease-resistant and cell-permeable double-stapled peptides	Cromm *et al.*,2016
Rab8a and Pi3K	Rab8a-recruits Phosphatidylinositol 3-Kinase γ regulates signalling and cytokine outputs from endosomal Toll-like Receptors.	Wall *et al.*,2017
Rab8	Endocytic turnover of Rab8 controls cell polarization	Vidal-Quadras *et al.*, 2017
RAB5/RabF		
Rab5	Rab5 is grouped into two subtypes: plant-unique ARA6 also known as RABF1and the canonical RAB5 group ARA7 and RABHOMOLOG1 [RHA1], also known as RABF2b and RABF2a	Ueda *et al.*,2001, 2004; Ebine and Ueda, 2009
Rab5	Analysis of Rab5 family in *Mesembryanthemum crystallinum* with increased level of expression during early salt stress.	Bolte *et al.*,2005
Rab5 and glup4	Rice glup4 mutant suggests a role for the RAB5 in the biosynthesis of carbon and nitrogen storage reserves in developing endosperm	Doroshenk *et al.*, 2010
Rab5	GLUP4/Rab5a and GEF- GLUP6/GEF, participate in the intracellular transport of the proglutelin from the Golgi apparatus to the PSV	Fukuda *et al.*,2011a, 2013
Rab5 and Dynamin	Role of Rab5 and Dynamin -dependent endocytosis of a Ca2+ activated K+ channel, KCa2.3.	Gao *et al.*,2012a
Rab5/ARA6	Endosomal trafficking pathway is regulated by RAB5/ARA6 in plants	Ebine *et al.*,2012
Rab5 and Rab5GEF	Rab5 GEF is essential for intracellular transport of the proglutelin from the Golgi apparatus to the protein storage vacuole in rice endosperm.	Fukuda *et al.*,2013
OsRAB5A and OsVPS9A	OsVPS9A and OsRAB5A to regulate post-Golgi dense vesicle-mediated storage protein trafficking to the protein storage vacuole in rice endosperm cells.	Liu *et al.*,2013
Rab5-ARA6	Molecular and biochemical analysis of ARA6/Rab5 in green algae	Hoepflinger *et al.*,2013

Rab5 , SAND-CCZ1	SAND/MONENSIN SENSITIVITY1 and CALCIUM CAFFEINE ZINC SENSITIVITY1 (CCZ1) act as effector of RAB5 and GEF for Rab7	Cui *et al.*,2014; Ebine *et al.*,2014; Singh *et al.*,2014
MiRab5	Molecular characterization and expression analysis of a mango MiRab5 in *Mangifera indica.*	Liu *et al.*,2014c
CaARA7 (RAB5)	Molecular analysis and localization of CaARA7 (RAB5) GTPase in Characean Algae	Hoepflinger *et al.*,2015
Rab5/ARA6 and QQS	AtRAB5/ARA6 is required for starch and sugar homeostasis through the function of Qua-Quine Stach gene (*QQS*), and the proliferation of *Pseudomonas syringae* pv. tomato DC3000 was repressed in *AtRabF1* knockout mutant	Tsutsui *et al.*,2015
Rab5	AtRab5 and its interacting partner ENDOSOMAL RAB EFFECTOR WITH PX-DOMAIN (EREX) regulates trafficking to protein storage vacuoles	Sakurai *et al.*,2016
Rab5	Enrichment of Phosphatidylethanolamine in viral replication compartments via co-opting the endosomal Rab5 by a Positive-Strand RNA Virus.	Xu and Nagy, 2016
RABF1 /ARA6	RABF1 and the membrane trafficking pathway at the interface between plants and obligate biotrophic pathogens	Inada *et al.*,2016
Rab5A	Rab5A gene of marine fish, large yellow croaker (*Larimichthys crocea),* and its response to the infection of *Cryptocaryon irritans*	Han *et al.*,2016
RabF1 and RabF2	RAB5-ARA6/RABF1 is localized to the specialized membrane that surrounds the haustorium, the extrahaustorial membrane (EHM), formed by the *A. thaliana*-adapted powdery mildew fungus *Golovinomyces orontii* and RAB5-ARA7/RABF2b was also localized to the EHM and endosomal SNARE	Inada *et al.*,2016
Rab5	Rab5 regulate different modes of endocytosis in *Leishmania*	Rastogi *et al.*,2016
Rab5a	Purification and characterization of TcRab5a from medicinal plant *Tinospora cordifolia*	Amir *et al.*,2016
Rab5b	Plasmodium Rab5b is secreted to the cytoplasmic face of the tubovesicular network in infected RBCs together with N-acylated adenylate kinase 2	Ebine et al.,2016
RabF1	Role of AtRabF1/ARA6 in salt stress and Dark-Induced Senescence (DIS)	Yin *et al.*,2017
Rab5	Membrane localization and dynamics of Geranylgeranylated Rab5 hypervariable region	Edler *et al.*,2017
Rab7/RabG		
RabG3b	AtRabG3b as a salicylic acid-responsive protein, transgenic RabG3bOX (over expression) and RabG3bCA (Constitutive active) plants displayed unrestricted hypersensitive programmed cell death (PCD) against a fungal toxin Fumonisin B1 and a fungal pathogen *Alternaria brassicicola*	Oh *et al.*,2005
PgRab7	Role of Plant Rab GTPase in stress tolerance in Pennisetum	Agarwal *et al*.,2008, 2009
RabG3b	At RabG3b in Pathogen Response and Leaf Senescence	Kwon *et al.*,2009
Rab7, Mon1-Ccz1 GEF	Mon1-Ccz1 GEF, Rab7/Ypt7 in yeast is recruited to endosomes	Kinchen and Ravichandran, 2010; Nordmann *et al.*,2010; Poteryaev *et al.*,2010; Yousefian *et al.*,2013; Singh *et al.*,2014; Li *et al.*,2015a
Rab7B3	OsRab7B3 enhances leaf senescence in transgenic rice	Pitakrattananukool *et al.*,2012
Rab7	Comprehensive genomic analysis Rab G proteins and their evolution	Klopper *et al.*,2012
RabG3b	AtRabG3b positively regulates autophagy and immunity-associated hypersensitive cell death	Kwon *et al.*,2013

CaRABF1	Biochemical and molecular characterization of CaRABF1 (CaARA6) in green algae *Chara australis*	Hoeplfinger *et al.,* 2013
Rab5, Rab7 and SAND	Rab5 effector SAND protein is necessary for Rab7 activation and in plant vacuolar trafficking	Singh *et al.,* 2014
Rab5, Rab7 and MON1-CCZ1	MON1–CCZ1 complex mediate Rab5 to Rab7 conversion in PVC to vacuole trafficking in plants	Cui *et al.,* 2014
Rab7, Mon1-Ccz1 GEF and PI3P	Mon1-Ccz1 GEF activates the Rab7 -Ypt7 via a longin-fold-Rab interface and associates with PI3P-positive membranes.	Cabrera *et al.*,2014
Rab7	Role of Rab7 in prostate cancer progression.	Steffan *et al.*,2014
Rab7	RAB7 controls melanoma progression by exploiting a lineage-specific wiring of the endo-lysosomal pathway	Alonso-Curbelo *et al.*,2014
Rab7/Ypt7, HOPS, V1-ATPase	Rab7-like Ypt7p, HOPS complex and V1-ATPase regulates lipid droplet dynamics in *Saccharomyces cerevisiae*.	Bouchez *et al.*,2015
Rab7	Multiple Roles of the Small GTPase Rab7 in trafficking	Guerra and Bucci, 2016
RabG3f	NbRABG3f is involved in Bamboo mosaic virus infection in *Nicotiana benthamiana*	Huang *et al.*,2016a
Rab7	Phospho-regulation of Rab7 during endosome maturation	Shinde and Maddika, 2016
Rab7	Use of site-directed mutagenesis, *in vivo* electroporation and mass spectrometry in search for determinants of the subcellular targeting of Rab7b paralogue in *Paramecium octaurelia*	Wyroba *et al.*,2016
Rab7, ARL8b	Rab7 effector PLEKHM1 binds Arl8b to promote cargo traffic to lysosomes.	Marwaha *et al.*,2017
Rab7	Rab7 regulates clathrin-mediated endocytosis and salinity stress resistance in peanut.	Sui *et al.*,2017a,b
Rab7	Retromer-driven membrane Tubule formation depends on the Rab7/Ypt7-interacting retromer complex, consisting of the sorting nexin dimer (SNX-BAR) and the trimeric cargo selection complex (CSC).	Purushothaman *et al.*,2017
RAB6/H		
Rab6	Rab6 coordinates a novel Golgi to ER retrograde transport pathway in live cells	White *et al.*,1999
Rab6, mint3	Rab6 interacts with the mint3 adaptor protein	Teber *et al.*,2005
AtRAB-H1b AtRAB-H1c	AtRAB-H1b and AtRAB-H1c target reporter proteins to the Golgi when expressed in *Nicotiana tabacum* and *Arabidopsis thaliana*	Johansen *et al.*,2009
Rab6	Rab6 regulation of the kinesin family KIF1C motor domain contributes to Golgi tethering	Lee *et al.*,2015
Rab6a	Involvement of Rab6a in organelle rearrangement and cytoskeletal organization during mouse oocyte maturation.	Ma *et al.*,2016
Rab6a and Myosin-IIA	Role of Rab6a and phosphorylation of non-muscle myosin IIA tailpiece in alcohol-induced Golgi disorganization	Petrosyan *et al.*,2016
Rab6a	OsRab6a, is involved in the regulation of iron homeostasis in rice	Yang and Zhang, 2016
Rabs in Other Organisms		
RAB	Analysis of 52 Rab GTPases from channel catfish and their involvement in immune responses after bacterial infections.	Wang *et al.*,2014a
RAB	Rab GTPases are essential for membrane trafficking-dependent growth and pathogenicity in *Fusarium graminearum*.	Zheng *et al.*,2015
RAB	Analysis of Rab GTPases in Archaea	Surkont and Pereira-Leal, 2016
Small GTPases	Role of small GTPase MoSec4 in the vegetative development and pathogenicity by regulating the extracellular protein secretion in *Magnaporthe oryzae*	Zheng *et al.*,2016
RAB	Role of Rab GTPases in membrane trafficking pathway in plants and obligate biotrophic pathogens	Inada *et al.*,2016

GAP		
AtAGD7	Overexpression of AtAGD7 causes relocation of Golgi localized proteins to the ER and inhibits protein trafficking in plant cells	Min *et al.*,2007
GAP	Role of GAPs in cellular logistics	Kahn, 2011
Rab GEFs and GAPs	Role of Rab GEFs and GAPs	Barr and Lambright, 2010
Rab GAPs	Role of TBC proteins as GAPs for mammalian Rab GTPase	Fukuda, 2011b
TBC-GAP, ARF and Rab1	Structurally distinct bacterial TBC-like GAPs link ARF GTPase to Rab1 inactivation to counteract host defence	Dong *et al.*,2012
TBC-Rab GAP	Evolution of Tre-2/Bub2/Cdc16 (TBC) Rab GAP GTPase activating proteins	Gabernet-Castello, 2013
RabGAP TBC1D15	TBC1D15 Family from *Chiloscyllium plagiosum* as GAP for Rab7	Li *et al.*,2015a
GAP and AtRanGAP	GAP activity is required for AtRanGAP cellular and developmental function	Boruc *et al.*,2015
GAP-TBC1D14	TBC1D14 GAP sets the TRAPP for ATG9 for autophagy	Lamb *et al.*,2016
GAPs and GTPases	Small GTPases and their GAPs	Mishra and Lambright, 2016
GAP	Role of phospholipases as GAPs in plants	Pandey, 2016
GEF & GDI		
RabGEF	RabGEFs are a major determinant for specific Rab membrane targeting	Blümer, 2013
GEF	GEF-effector interactions	Jackson, 2014
OsRhoGDI2	Expression patterns of OsRhoGDI2 from *Oryza sativa* during development and abiotic stresses	Huang *et al.*,2016b
AtRho-GDI	AtRhoGDIs are critical for cellular homeostasis of pollen tubes	Feng *et al.*,2016
GEF	Pull-down procedure for the identification of unknown GEFs	Koch *et al.*,2016
RabGEF1	RABGEF1 regulates keratinocyte-intrinsic signalling to maintain skin homeostasis.	Marichal., 2016
RAB GEF	Artificial membrane tethering for characterizing activation of small GTPases by their GEFs on membrane	Peurois *et al.*,2017
RABGGTase		
Rab GGTase RGTB1	Rab GGTase mutants RabGTB1 beta subunit are constitutively photomorphogenic, and has shoot growth and gravitropic defects.	Hala *et al*., 2010
AtRab GGTase	Redundancy and broad substrate specificity of AtRABGGTase (Arabidopsis Rab Geranylgeranyltransferases)	Shi *et al.*,2016
RAN		
RanGAP1	RanGAP1 as marker of the Arabidopsis cell division plane.	Xu *et al.*,2008
RAN2	Overexpression of OsRAN2 in rice and Arabidopsis renders transgenic plants hypersensitive to salinity and osmotic stress.	Zang *et al.*,2010
RAN1	OsRAN1 is involved in plant cold resistance in rice	Xu and Cai, 2014
LlaRan	Overexpression of Ran gene from *Lepidium latifolium* L. (LlaRan) renders transgenic tobacco plants hypersensitive to cold stress	Sinha *et al.*,2014
Ran	Spatial organization of RAN pathway by microtubules in mitosis.	Oh *et al.*,2016
PdRanBP	Role of PdRanBP regulates vascular tissue development in poplar	Li *et al.*,2016d
Ran	Quantum mechanics/ molecular mechanics studies on Ran GTPase and role of Magnesium	Mooney *et al.*,2016
AtRan1	AtRAN1 regulates vegetative growth and stress tolerance	Xu *et al.*,2016a
VvRan1	Stress Responses of *vvran1* in the Straw Mushroom *Volvariella volvacea*	Yan *et al.*,2016
ROP		
ROP	ROP GTPase-switch controls multiple developmental processes in Arabidopsis	Li *et al.*,2001
ROP10	Plasma membrane-associated ROP10 is a specific negative regulator of ABA in Arabidopsis	Zheng *et al.*,2002a
ROP	Theoretical model for AtROP localisation by auxin in arabidopsis root hair cells	Payne and Grierso, 2009

VvROP	Cellular localisation of VvRops and VvRabA5e and is developmentally regulated in grape berries.	Conéjéro *et al.*,2010
ROP1, ROP GEF, AtPRK2	AtPRK2 promotes ROP1activation via RopGEFs in the control of polarized pollen tube growth	Chang, *et al.*,2012
ROP	Rho signalling from plant ROP/Rac GTPases.	Craddock *et al.*, 2012
ROP6	ROP6 interacts with NFR5 and is involved in nodule formation in *Lotus japonicus*	Ke *et al.*,2012
ROP	New insights into Rho signalling from plant ROP/Rac GTPases	Craddock *et al.*,2012
ROP6	AtROP6 functions in developmental and pathogen response pathways.	Poraty-Gavra *et al.*,2013
AtROP3	AtROP3 GTPase contributes to polar auxin transport and is important for embryogenesis and seedling growth in Arabidopsis	Huang *et al.*,2014
ROP/RAC5	RISAP is a TGN-associated RAC5 effector regulating membrane traffic during polar cell growth in tobacco	Stephan *et al.*,2014
ROP	ROP GTPase regulation of auxin transport in Arabidopsis.	Ren and Deshu 2015
ROP6 and CHC1	Clathrin heavy Chain1 is associated with ROP6 and is involved in nodule formation *Lotus japonicus*	Wang., *et al.*,2015
ROP GAP	Ethylene negatively regulates transcript abundance of ROP-GAP and affects apoplastic ROS homeostasis in epicarps of cold stored apple fruits.	Zermiani *et al.*,2015
ROP	SPIKE1 activates ROP GTPase to modulate petal growth and shape	Ren *et al.*,2016
ROP6	ROP6 is involved in root hair deformation induced by Nod factors in *Lotus japonicus*	Ke *et al.*,2016
ROP2 TOR and Auxin	ROP2 binds and promotes activation of target of rapamycin, TOR, in response to auxin	Schepetilnikov *et al.*,2017
ROP2 and MAP18	The Microtubule-Associated Protein MAP18 affects ROP2 GTPase activity during root hair growth.	Kang *et al.*,2017
ROP2-RIC1	AtROP2-RIC1 pathway fine-tunes microtubule reorganization for salt tolerance in Arabidopsis	Li *et al.*,2017a
MIRO GTPase		
MIRO	The mitochondrial Ras-related GTPase Miro (Miro GTPase) in metazoan kingdom.	Yamaoka and Hara-Nishimura, 2014
SAR GTPase		
ARF	Genes encoding ADP-ribosylation factors (ARF) in *Arabidopsis thaliana* L. Heyn.; genome analysis and antisense suppression.	Gebbie *et al.*,2005
ARF6 and JIP4	ARF6 Interacts with JIP4 to control a motor switch mechanism regulating endosome traffic in cytokinesis	Montagnac *et al.*,2009
Sar1	Golgi membrane dynamics after induction of a dominant-negative mutant of Sar1 GTPase in tobacco.	Osterrieder *et al.*,2010
Sar1	Sar1 as a Novel Regulator of ER-Mitochondrial Contact Sites.	Ackema *et al.*,2016
Sar1	Sar1 GTPase activity is regulated by membrane curvature	Hanna *et al.*,2016
ARF and ARL		
AtARF1	AtARF1 plays a critical role in intracellular trafficking and maintenance of ER morphology in Arabidopsis	Lee *et al.*,2002
ARF1	Arf1 GTPase plays roles in the protein traffic between the ER and the Golgi apparatus in tobacco and Arabidopsis cultured cells	Takeuchi *et al.*,2002
ARF1	ARF1p controls the sequence-specific vacuolar sorting route to the lytic vacuole	Pimpl et al ., 2003
AtARF1	Dissection of AtARF1 function in epidermal cell polarity	Xu and Scheres, 2005
ARF, RAB	Membrane recruitment of effector proteins by ARF and Rab	Kawasaki *et al.*,2005
ARF	ARF proteins their roles in membrane traffic and beyond.	D'Souza-Schorey and Chavrier, 2006
ARF-GEF	ARF-GEF acting at the Golgi and in selective endocytosis in polarized plant cells.	Teh and Moore, 2007
ARF GEF	Use of fluorescence imaging-based screen to identify ARF GEF component of early endosomal trafficking	Tanaka *et al.*,2009

ARF	ARF machinery mediates endocytosis in plant cells	Naramoto *et al.*,2010
ARF	Analysis of Arf GTP-binding protein function in cells	Cohen *et al.*,2010
ARFA1b/1c, ROR2 Syntaxin	The multivesicular body (MVB)-localized ARFA1b/1c GTPase is important for callose deposition and ROR2 syntaxin-dependent pre-invasive basal defense in barley.	Böhlenius *et al.*,2010
ARF and RAB	Molecular characterization and expression analysis of 4 Rab and 2 Arf GTPases in the latex of *Hevea brasiliensis*.	Qin *et al.*,2011
ARF1	ARF1 localizes to the Golgi and the trans-Golgi network.	Robinson *et al.*,2011
ARF1 and ARF3	ARF1 and ARF3 are required for the integrity of recycling endosomes and the recycling pathway	Kondo *et al.*,2012
SAR and ARF	Expression of ARF1 and SAR1 during development and the de-etiolation process	Keskin *et al.*,2012
ARF4, ASAP, Rab8, Rab11	ARFGAP- ASAP1 provides a platform to regulate Arf4- and Rab11-Rab8-mediated ciliary receptor targeting	Wang et al.,2012
ARF, ARL	Role of Arf/Arl small GTPases in cilia and ciliopathies	Li *et al.*,2012
ArfGEF GBF-1	ArfGEF GBF-1 Is Required for ER Structure, Secretion and Endocytic Transport in *C. elegans*	Ackema *et al.*,2013
SAR and ARF	Role of ARF1 and SAR1 GTPases in endomembrane trafficking in plants	Keskin, 2013
Sec7 Arf-GEF	Regulation of the ARF GEF to direct traffic at the TGN.	McDonold *et al.*,2014
SAR and ARF	Molecular mechanisms of Sar/Arf GTPases in vesicle trafficking in yeast and plants	Yorimitsu *et al.*,2014
NtGNL1a	NtGNL1a ARF-GEF acts in endocytosis in tobacco cells	Jelínková *et al.*,2015
ARL	ARL5 contributes to endosome-to-Golgi traffic by aiding the recruitment of the GARP complex to the Golgi	Rosa-Ferreira *et al.*,2015
ARF1 and Memb11	Qb-SNARE Memb11 interacts specifically with Arf1 in the Golgi apparatus of Arabidopsis	Marais *et al.*,2015
ARF/ARL/ SAR	Plasmids for the expression or knockdown of human ARF Family GTPases (ARF/ARL/SAR) and their coexpression in bacteria with N-myristoyltransferases,	Kerr and Kahn, 2015
ARF and RAB	Role of Rab and Arf GTPases in endosomal trafficking and cell surface homeostasis.	Donaldson *et al.*,2016
ARF	Role of Arf GTPases in fungal morphogenesis and virulence	Labbaoui *et al.*,2017
SAR1 and ARF1	Role for Sar1 and ARF1 GTPases during Golgi biogenesis in *Trypanosoma brucei*	Yavuz *et al.*,2017

1.9 Rab11/RabA GTPase and tomato fruit ripening

The role of cell wall metabolism and characteristic functions and different roles of Rab GTPase in plants have been extensively reviewed by Lycett (2008). The diagram in fig. 1.4 shows the possible pathways in which Rab GTPases are involved. The mammalian Rab11 GTPases are associated mainly with endocytosis and recycling endosomes (Zerial and McBride, 2001) but evidence from plants suggested that some groups are associated with transport to the cell membrane (Ueda *et al*., 1996; Lu *et al*., 2001; Inaba *et al*., 2002; de Graaf *et al*., 2005; Lycett, 2008).

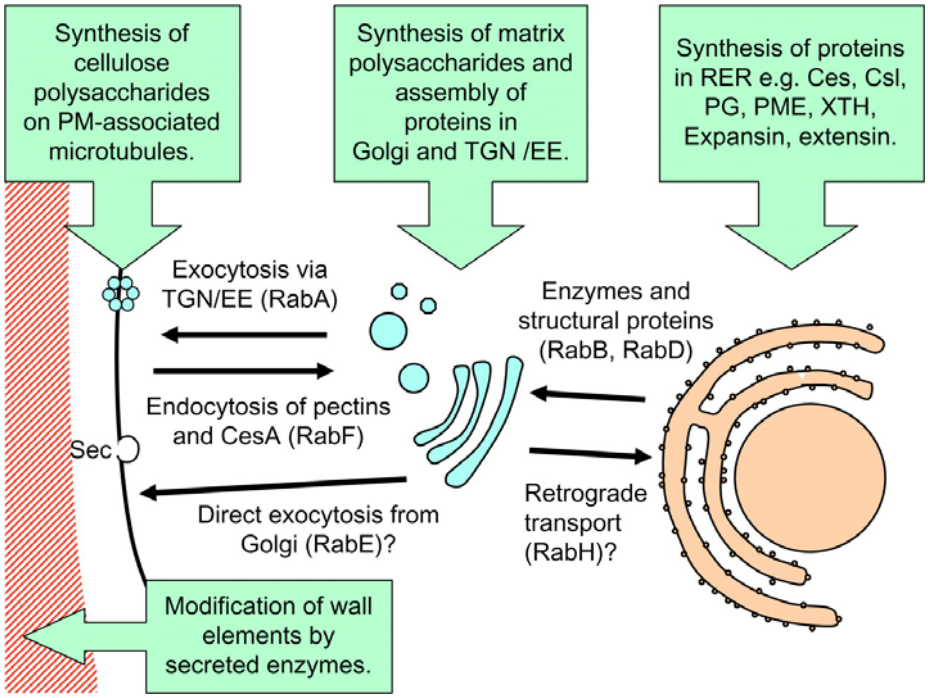


Fig. 1.4. Intracellular Vesicle Trafficking by Rab GTPases in plants. (Source: Lycett, 2008). A schematic representation of the trafficking pathways to and from the cell wall. Pathways are shown as arrows with probable cargoes indicated. The Rab GTPases probably involved at each step are indicated in parentheses. Pathways to and from the vacuole are not shown. TGN/EE refers to the trans-Golgi network and early endosomal compartments which now seem to partially overlap in plant cells and possibly function in both endocytosis and exocytosis.

Elements of the secretory pathway, such as Rab GTPases, have been shown to be expressed in a ripening related manner in fruit (Loraine *et al*., 1996; Zainal *et al*., 1996; Zegzouti *et al*., 1999; Lu *et al*., 2001; Park *et al*., 2006). Three Rab1 proteins from tomato are expressed strongly in fruit (Loraine *et al*., 1996). One of these (*LeRab1C*) is expressed most strongly in expanding green fruit and the other two (*LeRab1A* and *LeRab1B)* more strongly in ripe fruit. The corresponding proteins differed in their ability to interact with geranylgeranyl transferases (GGTase) from yeast, mammalian cells and plant cells. Thus, though they are all the same class of protein, presumably regulating the same step in the secretory pathway and they are all expressed in fruit, there may be subtle differences in their roles. *Rab8* and *Rab11* genes have also been found to be preferentially expressed in tomato fruit (Zegzouti *et al*., 1999; Lu *et al*., 2001).

It has been suggested that the Rab GTPases regulate secretion of the enzymes that modify the cell wall to bring about fruit softening (Loraine *et al*., 1996; Zainal *et al*., 1996). This has been investigated for the Rab11 class, first identified in ethene-treated mango fruit mesocarp (Zainal *et al*., 1996). A tomato

orthologue (*LeRab11a*) was silenced by transformation with antisense constructs. The transgenic tomato plants had fruit that remained firm for a very long period and it was shown that the levels of extracted polygalacturonase and pectinesterase were reduced in these fruits (Lu *et al*., 2001).

The *Rab11* antisense tomato plants also showed distinct physiological and developmental differences, including altered floral organ identity, highly branched inflorescences, rumpled leaves, ectopic shoots from leaves, determinate growth, reduced apical dominance and altered ethene production. These effects are reminiscent of developmental abnormalities and altered hormone levels, where Rab11 GTPases have been over expressed (Kamada *et al*., 1992; Sano *et al*., 1994; Aspuria *et al*., 1995) and are not likely to be due to altered trafficking to the cell wall.

The gene *LeRab11a* has been shown to be expressed much more strongly in expanding green fruit than in ripe fruit and the reduction in pectinesterase and β-galactosidase levels was much greater in green fruit. In a more recent study using tobacco leaf protoplasts, LeRab11a-GFP fusions were shown to be associated with the *trans*-Golgi network. Dominant negative mutant versions of the *Rab* gene also inhibited trafficking of secreted GFP. Furthermore, inhibiting the SYP121 pathway as well as Rab11a caused a greater inhibition of trafficking than just the *LeRab11a* dominant negative mutant alone, whereas inhibition of SYP122 did not. This indicates that SYP122 and Rab11a are probably involved in the same pathway but SYP121, which has been implicated in ABA responsive secretion may be involved in a different pathway (Leyman *et al*., 2000). However, several Rab GTPases have been shown to be expressed in a range of actively growing or highly secretory tissues (Cheung *et al*., 2002; Lu *et al*., 2001) and inhibition of LeRab11a did not inhibit overall growth (Lu *et al*., 2001). It is also possible that different proteins and polysaccharides destined for the cell wall are delivered by different vesicles and support for this has recently emerged (Leucci *et al*., 2007; Lunn *et al*., 2013).

Most macromolecules that are secreted from the cell are transported by vesicles through the endomembrane system. Polysaccharides are synthesised at the cell membrane or in the Golgi apparatus and secreted proteins are usually synthesised on the endoplasmic reticulum (ER) and transported via the Golgi apparatus and the *trans*-Golgi network (TGN) on their way to the plasma

membrane (Hanton and Brandizzi, 2006). Within this trafficking system, transport is by vesicles that bud from one organelle and fuse with another and these may move in an anterograde or retrograde direction. The fusion of the correct vesicle with the correct target membrane is thought to be regulated by Rab GTPases and in mammalian systems there are 41 sub classes of these, each of which regulates docking of different vesicles with their target membranes (Zerial and McBride, 2001).

1.10 Aim of the Project

To investigate the role of Rab GTPases in secretion of cell wall factors associated with softening and thereby offer a way of manipulating texture without an effect upon flavour and colour development. The objective is to find out which Rabs and/or other elements of the trafficking machinery are expressed in fruit, especially in a ripening related manner and in other parts of the plant where cell wall modification is occurring.

1. *In silico* analysis of Rab GTPases in tomato.
2. Classification of the tomato Rab GTPases into different classes.
3. Screen the expression profiles of the different Rab GTPases in tomato.
4. Collect the developmental series in wild type and transgenic tomatoes.
5. Molecular characterization and phenotypic characterization of transgenic plants.
6. Textural analysis: compression and penetration test on fruits.
7. Generate the relative expression profiles by qRT-PCR.
8. To find other proteins involved in the intracellular vesicle/membrane trafficking in tomato by *in silico* analysis.

2. Materials and Methods

2.1 Plant Material

The seeds of tomato cultivar Ailsa Craig (AC++) and the seeds of transgenic tomato lines of antisense *Le Rab11a* were kindly provided by Dr. G. W. Lycett.

Lu *et al*., (2001) used *Agrobacterium* mediated genetic transformation to transform tomato with *Rab11a* gene in antisense orientation in the Ailsa craig (AC++) background. Cotyledons were used as explants for co-culturing. Three antisense constructs were generated containing one half length (pGATR), and two full length (pBINTR and pBINDR) antisense *Le Rab11a* gene under the control of CaMV35S promoter.

Lu *et al*., (2001) obtained several independent transformed tomato lines which were named individually as T for transformed e.g. T_1, T_2, T_3 etc. Here 1, 2, 3,…,n, represent individual transformed plants which were obtained by them. Out of several independent confirmed transformed tomato lines T_1, T_3 and T_5 were selected as *Le Rab11a* was down-regulated in these lines in fruits. The further generation of which were named by Lu *et al*., (2001) as R_1. Thus, the plants obtained by sowing R_1 generation seeds were called R_2 and likewise for the subsequent generations. This is in contrast with the actual, normally accepted nomenclature for naming the transgenic events and subsequent generations.

The normally accepted term for any independent transformed plant obtained from tissue culture is T_0 generation and the progeny of T_0 is named T_1 and like wise for the next subsequent generations. As far as this investigation is concerned the following should be used as $T_{(event)}$ $R_{(Generation)}$ descriptor for follow-up of the subsequent generation of the tomato antisense *Rab11a* lines.

The present investigation is based on the analysis of the progeny of T_1 event and its third generation (R_3). The transgenic plants of T_1 R_3 were selected for the present study and for the control TMV sensitive Ailsa craig (AC++) was used. The TMV resistant derivative of Ailsa Craig has different ripening characteristics due to the ripening related genes on the introgressed chromosome fragment. The TMV resistant line shows little change in softening as the fruit ripens so the expected phenotype would have been more difficult to see.

2.2 Harvesting and Extraction of Seeds from the Transgenic Lines

The nine plants of T_1 R_3 which were being grown in the glasshouse were selected for harvesting the fruits and extraction of seeds. The fruits were harvested from each individual plant separately and properly labelled. The seeds were extracted from the ripe fruits by squeezing. The seeds were collected separately and were treated with 0.1N HCl and were then left overnight. The seeds were washed thoroughly with water and were left for drying on a blotting paper. Finally the dried seeds were stored in a brown paper bag and labelled properly.

2.3 Sowing of Seeds of the Progeny of Rab11 Antisense Lines

To test the Mendelian segregation ratio and to test the homozygosity of the transgenic lines, twenty four seeds from each of the nine transgenic lines were sown (24x9=216). Along with this 20 plants of AC++ were grown as control for the experiment. The potting mix M3 was used, which was mixed with perlite. Every pot was filled with the potting mix up to the rim; seeds were sown in every pot. After sowing seeds, all the pots were watered thoroughly. The pots containing the wild type seeds were labelled with white stick whereas the pots with transgenic seeds were labelled properly with orange stick. Along with this the cuttings from the original R_3T_1 lines were taken and grown in the pots.

When the plants reached optimum height they were transferred to larger pots and then transferred to the main glasshouse.

2.4 Plant DNA Extraction

The fresh leaf samples from the nine transgenic lines and AC++ control plants were collected. The leaf samples were collected early in the morning. A clean pair of forceps was used to collect the fresh and young leaves from the control and transgenic lines. The 96 well plate box was labelled. During the collection of the samples the box containing the samples was kept on ice.

2.4.1 Preparation of Extraction Buffer:

Speed DNA Extraction Buffer

1M Tris HCl (pH 7.5)	20 ml
4 M NaCl	6.25 ml
0.5 M EDTA	5.00 ml
10% SDS	5.00 ml

The individual solution were made and mixed according to the proportions and the final volume of 100 ml was made with the sterile distilled water.

2.4.2 The speed DNA extraction protocol

The leaf samples were placed in the individual tubes of the 96 well plates (Qiagen no. 19560). The box was kept on ice during the sample and addition of buffers. For storage the samples were kept for long duration in a -20° C freezer for long term storage. To every single well a single 3 mm stainless steel ball was added. An aliquot of 600 µl of the extraction buffer was added in each well. The 96 well-plate was then covered and sealed with the micro-tube caps (Qiagen No. 19566). The box was sealed to avoid any leak and then the box was kept on shaker for 3 minutes in a ball box mill. The box was taken out and was then centrifuged at 5600 g for 20 minutes.

The box was taken out after 20 minutes. From every well, 300 µl of supernatant was taken with the help of multi-channel digital pipette (long narrow pipette tips such as 1250 µl matrix pipette tips). The supernatant was transferred to a fresh plate (Thermo scientific # AB 1127 and AB 0675) containing 300 µl of 100% iso-propanol. It was incubated at room temperature for 3 minutes. And then it was centrifuged at 5600 g for 20 minutes. The supernatant was removed by aspiration, enough care was taken to aspirate from the corner of the well with the plate tilted at 45° angle. To these plates, 100 µl of 70% ethanol was added and it was mixed well by shaking vigorously for 20 minutes. The supernatant was removed by aspiration. The plates were dried under desicator for 30 minutes. The DNA pellet was re-suspended in 0.1 X TE buffer (20 µl). The plates were then stored in a -20 C freezer.

2.5 PCR

PCR was used to amplify the gene of interest from the total genomic DNA isolated from the progeny of the transgenic lines. The primers specific to *Le Rab11a* gene were used for PCR. The primer details are given below along with expected amplified product size.

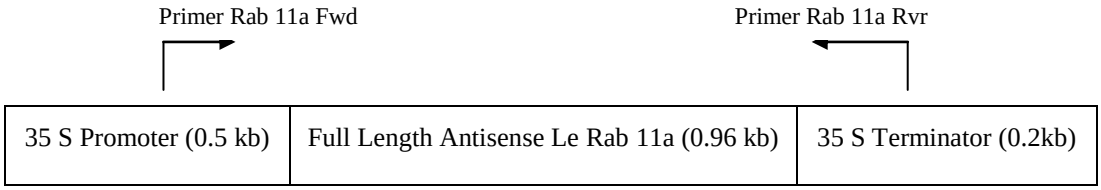
Gene of interest and the primer information

Gene of interest: *Le Rab11a*

Primer1: *Rab 11a* Fwd: GGA CCC CCA CCC ACG AGG A 19 bases

Primer2: *Rab 11a* Rvr : GGG GTT GGA TTT GCA ACC 18 bases

Expected band size: 0.96 kb (960 bp)



The PCR reaction was carried out in a 20 µl volume. Megamix Blue double PCR master-mix was used for the reaction.

Solution	**Volume**
2x MMDB Megamix Blue Double	10 µl
Primer 1 LeRab11a Fwd	0.5 µl
Primer 2 Le Rab11a Rvr	0.5 µl
Template DNA	1.0 µl
Distilled Water	8.0 µl
Total	20 µl

The amplification cycles were as under

	Cycle	Temperature	Time
	1	95° C	3 min
	35	94° C	30 Sec
		50° C	60 sec
		72° C	2 min
Final Extension		72° C	10 min
		Hold	4

2.6 Agarose gel electrophoresis

The PCR product was run on a 1% agarose gel and separated by electrophoresis. Agarose (1 gm) was dissolved in 100 ml of TAE buffer. Agarose was added to 250 ml conical flask to which 100 ml of TAE buffer was added. It was heated in the oven for 2 min to dissolve the agarose. It was then cooled to touch at 50 ° C. Ethidium bromide 1% (4 µl) was added to the flask. It was then poured into the casting tray and the comb was set into the tray. The gel was allowed to set. The combs were removed carefully from the gel to avoid any damage to the wells. The tank was filled with the TAE buffer. The PCR products samples were loaded into the wells carefully with 1 Kb DNA ladder (Promega) along with the positive (Plasmid DNA) and the negative control Wild type (Non transgenic, DNA sample from wild Type AC++). It was then run at 80 V for 40 min and was analysed in the Gel Doc system.

2.7 RNA isolation

2.7.1 Preparation of Buffer

The following buffers and solutions were prepared according to standard procedures and protocols.

Extraction Buffer:	**250 ml**
2% CTAB (hexadecyltrimethylammonium bromide)	5 g
2% PVP (Polyvinylpyrolidone K 30)	5 g
100mM Tris-HCl (pH 8.0)	25 ml of 1M Tris HCl
25mM EDTA (pH 8.0)	1.25ml of0.5M EDTA
2.0 M NaCl (mix, Heat to Dissolve)	29.22 g
2% β-mercaptoethanol	20 µl/ml

Chloroform

8M Lithium Chloride (made with DEPC treated H_2O and Autoclaved)

SSTE Buffer:	**250 ml**
1.0M NaCl	14.61 g
0.5% SDS	6.25 ml of 20% SDS
10 mM Tris-HCl (pH 8.0)	2.5ml 1mM Tris HCl
1mM EDTA (pH 8.0)	0.5ml of 0.5M EDTA

DEPC Treated H_2O,

Isopropanol (Stored @ -20°C),

70% Ethanol (Stored @ -20°C),

100% Ethanol (Stored @ -20°C)

2.7.2 RNA isolation Protocol (30ml Tube method)

First of all, β-mercaptoethanol was added to the extraction buffer (1.2ml/60ml/6 samples). One gram of ground tissue was taken in a mortar and to this 10 ml of extraction buffer was added and was ground vigorously for 30 seconds and 9 ml was transferred to 30 ml oakridge tube, which was then incubated at 65 degree for 10 minutes. This was extracted once with an equal volume of chloroform, and was vortexed for 15 seconds and then centrifuged for 10 min at 18514 g. The supernatant (7ml) was transferred to new tube and 0.8 volume of cold iso-propanol was added, mixed and left on ice for 5 minutes before a 10 minutes spin at 18514g. the pellet was washed with 70% cold ethanol and was centrifuged at 18514g for 10 min. The supernatant was discarded and the pellet was air dried for 2-5 minutes. The pellet was re-dissolved in 9ml of SSTE buffer which was incubated at 37 °C for 10 minutes. It was then extracted with 9 ml of chloroform, vortexed for 15 seconds and then centrifuged for 10 minutes at 18500g. Now 8 ml of supernatant was transferred to new tube and 6.4 ml of ice cold isopropanol was added, mixed and then left on ice and then centrifuged at 18500 g for 10 min. The pellet was then washed with 70% and 100% ethanol and centrifuged at 18500 g for 10 min. The pellet was air dried for 2-5 min. The pellet was dissolved in DEPC treated water and kept on ice. Now 200 micro liters of 8M LiCl was added to the supernatant and was mixed thoroughly and was incubated for 2 hours on ice and was then centrifuged at 15000g for 20 min at zero degree. The pellet was then washed with 70% and 100% ethanol and air dried. Now the pellet was re-suspended in 50 µl of DEPC treated water, vortexed and kept on ice for at least 2 hours before checking the concentration on Nanodrop spectrophotometer.

2.8 RT PCR and Quantitative real time PCR (qRT-PCR)

2.8.1 RT PCR

Primers for RT PCR were designed by using the Primer 3 software. While designing the primers the following parameters were used as standards for designing the effective primers for qRT-PCR.

1. T_m of primers: 58-60° C
2. GC Content : 30-80%
3. Primer length: 18-22 bp
4. Amplicon length: 50-150 bp

The best working primers for the specific genes were found by using the primer sequences as query for BLAST search in the TIGR DFCI-Tomato Gene Index database (http://compbio.dfci.harvard.edu/tgi/cgi-bin/tgi/gimain.pl?gudb=tomato). The original 5'-3' sequence of the forward primer and complimentary sequence of the primers of the 5'-3' sequence of reverse primer were used as query sequences. The forward and reverse primer sequences which gave the best and 100% hits only were used for the experiment. More details about the primers designing and parameters used are given in the sixth chapter.

For RT PCR the reaction was set up in 0.2 ml thin walled PCR tubes. For first strand cDNA synthesis the annealing of the oligo primer to RNA for priming was done by using random hexamer oligos (Promega C1181) at0.5 mg/ml. Total RNA was diluted to 0.1 µg/µl for the annealing incubation. The annealing step was done in a water bath incubator at 70° C.

The reaction for annealing of oligo primers to RNA was set up as follows

Total RNA (0.1µg/µl)	5 µl
Hexamer primers (0.5µg/µl)	1 µl
Water (RNase/DNase free)	9 µl
Final volume	15 µl

The reaction was set up in a thermocycler block at 70° C for 5 min and then the primer/RNA mix was kept on ice. For Reverse transcriptase reaction, the annealed RNA-primer mix was then extended by MMLV-Moloney Murine Leukemia Virus (Promega M170A) reverse transcriptase. Master mix was made for multiple reactions and added to the annealed primer–RNA mix.

Reagents	Vol	Conc.
MMLV-RT Buffer x5	5 µl	
dNTP mix (10mM each)	1.25 µl	0.5mM
RNase inhibitor (Promega N2511)	0.5 µl	25U
MMLV RT enzyme (Promega M170A)	1 µl	200U
Water (RNase/DNase free)	2.25 µl	
Final Volume	10 µl	

The master mix was mixed and vortexed gently and 10 µl of master mix was added to 15 µl of the primer-RNA mix (final volume 25 µl) and was incubated at room temperature for 10 min and then at 42° C for 1 hour. As a control for contaminant PCR product from genomic DNA and non mRNA transcripts the following reaction was set up side by side for control reaction.

Reagents	Vol	Conc.
MMLV-RT Buffer x5	5 µl	
dNTP mix (10mM each)	1.25 µl	0.5mM
RNase inhibitor (Promega N2511)	0.5 µl	25U
MMLV RT enzyme (Promega M170A)	No Enzyme	
Water (RNase/DNase free)	2.25 µl	

The cDNA thus obtained was analysed on gel and nanodrop spectrophotometer. After this RT reaction the cDNA stock was diluted 25 µl of cDNA and 75 µl of water to give 100 µl of stock solution. This was the first strand cDNA stock to be used for the qPCR. The quality of the cDNA was checked on Nanodrop spectrophotometer.

2.8.2 Quantitative real time PCR (qRT-PCR)

The quantitative real time PCR was done on Roche Lightcycler 480 Real-Time PCR system using the Lightcycler 480 SYBR Green master mix (Roche Cat:04707516001) in Roche Lightcycler 384 well plate (Roche Cat:04729749001). In every single well the reaction volume was 15 µl. The reaction was set up as given below.

Reagents	Vol.
SYBR Grenn Master mix (2x)	7.50 µl
Forward Primer (10 pmol/ml)	0.45 µl
Reverse Primer (10 pmol/ml)	0.45 µl
cDNA	5.00 µl
Distilled Water	1.60 µl
Total	15 µl

For each sample there were 3 biological replicates containing cDNA, three replicates for the non template control (NTC) containing only water and no cDNA and 3 replicates for non reverse transcriptase control (NRTC) which contained only RNA. In each well 10µl of master mix was added and 5µl of cDNA was added individually and likewise for NTC and NRTC 5 µl of water and RNA was added. The plate was then sealed using a transparent sealing foil. The plate was then centrifuged and then loaded to the Lightcycler. The amplification cycles were as under

	Cycle	Temperature	Time
Pre-incubation	1	95° C	5 min
Amplification	45	95° C	10 Sec
		60° C	15 Sec
		72° C	15 Sec

The melting curve analysis for checking the quality of primers was done to ensure that there is no contamination and there is no primer dimer formation in the reaction.

2.9 Texture analysis

The textural analysis of the transgenic fruits was done at different developmental stages. The fruits were harvested at mature green (MG), Breaker (B), B+5, B+10, B+15, B+20. The firmness of the fruits was measured by compression and penetration test.

2.9.1 Compression test: For compression test readings were recorded in two different ways, one single reading for polar and three reading for equatorial plane were recorded. For compression test the test speed was kept at 0.5 mm/sec and the test distance was kept as 4 mm. The force was measured in 'g'.

Settings for compression test

Mode:	Measure force
Pre test speed:	0.5 mm/sec
Test Speed:	0.5 mm/sec
Post test speed:	0.5 mm/sec
Distance:	4 mm
Trigger:	Auto-4g

2.9.2 Penetration test: In the penetration test the depth of penetration was measured under a constant load. The probe was made to penetrate into the fruits and the force necessary to achieve a certain penetration depth or the depth of penetration in a specified time, under defined conditions, was measured and used as an index of firmness for measuring the textural property of the fruits. The TA.XT2 analyzer was used to perform the test using BS757 0.5 diameter Delrin (2mm cylinder probe) under the following settings

Mode:	Measure force
Pre test speed:	0.5 mm/sec
Test Speed:	0.5 mm/sec
Post test speed:	0.5 mm/sec
Distance:	10 mm
Trigger:	Auto-4g

2.10 *in silico* analysis of Rab GTPases

The *in silico* analysis for the Rab GTPases in tomato was done using the bioinformatics tools and the databases available online. The analysis of the publicly available database offers a cost effective and rapid approach to elucidate genome and transcriptome of an organism. The expressed sequence tags (EST) and their consecutive assembly results in the optimization of the tentative Conesus (TC) sequence information. The EST represents the short, unedited and randomly selected single pass reads of the sequences derived from the cDNA libraries which provide an alternative to whole genome sequencing. This analysis enables the gene discovery, complete genome annotation, aids in identification of the structure of the gene and proteins along with enabling the validity of the alternate transcripts.

The databases used for *in silico* analysis include

1. TIGR-DFCI Tomato gene index (http://compbio.dfci.harvard.edu/cgi-bin/tgi/gimain.pl?gudb=Tomato),
2. Sol genomics network, SGN (http://solgenomics.net/) and
3. Tomato functional genomic database, TED (http://ted.bti.cornell.edu/).

The first step is data mining, which relates to the mining of the publicly available databases for finding the sequence information to generate a customized database of the all the DNA or protein sequence of interest. The pattern based searching of the database such as PSI-BLAST is useful tool to search sequences of interest (Altschul *et al.*, 1990). The databases were screened for the genes coding for Rab GTPase in tomato using BLAST search.

The first TGI release for tomato was on 12 March 1999. The BLAST 2.0 (Wu-BLAST) searches were carried out with TIGR DFCI Tomato Gene Index (LGI) Release 11, 12 and 13 during the course of the research and present study. Table 2.1 shows the increase in the number of unique sequence from TGI release 11 to 13. The table highlights the number of expressed sequence tags (ESTs) which are the partial, single pass sequences from either end of cDNA clones, Expressed Transcript (ET) and Tentative Consensus (TC) which are assemblies of the non human ESTs. There are 28167 confirmed TCs. Table 2.2 highlights the increased

number of ESTs representing the TC in TGI. Thus, the database was updated 3 times from release 11 to last release 13 (April, 2010).

Table 2.1 Tomato Gene Index Release: Number of unique Sequences

Release	Number of unique sequences	TCs	Singleton ESTs	Singleton ETs	Total
Release 13.0 April 13, 2010		28167	24139	196	**52502**
Release 12.0 July 15, 2008		25764	20884	201	**46849**
Release 11.0 June 21, 2006		21550	19684	191	**41425**

scale:

represents 10000> sequences

key:

number of TCs

number of singleton ESTs and ETs

Table 2.2 Distributions of input EST and ET Sequences in TIGR DFCI TGI

Release	Distribution of input sequences		in TCs	in singletons	Total
Release 13.0 April 13, 2010	ESTs		275661	24139	**299800**
	ETs		2793	196	**2989**
Release 12.0 July 15, 2008	ESTs		309512	20884	**330396**
	ETs		2788	201	**2989**
Release 11.0 June 21, 2006	ESTs		194263	19684	**213947**
	ETs		1852	191	**2043**

scale:

represents 100000 sequences

key:

number of sequences found in TCs

number of sequences found in singletons

Once the database for tomato Rab GTPase was updated on the basis of the latest release, the information was parsed and confirmed with the next database. Thus, it was again compared on 3x2 counts with the new SGN unigene builds with their subsequent release of new updates. The DNA and Protein sequences obtained from TGI were used for exhaustive BLAST (BLASTP; Altschul *et al*., 1997) searches and then used to screen the SGN database to obtain SGN Unigene ids.

According to the SGN website the unigene builds represent "minimally redundant" collections of expressed genes in Solanaceous species, constructed from EST/cDNA sequence. The unigene set is obtained from EST data by assembling together, in contigs, EST sequences which are ostensibly fragments of the same gene.

These SGN Unigene ids and the DNA and protein sequences were used as query sequences to find the probe ids from the TED database. After obtaining the probe id information for every sequence of Rab GTPase the next objective was expression profiling. The expression profiles were generated by using the probe ids obtained from SGN database, which were fed into the TED database. The tools available for clustering from TED website were used to generate the Heat maps using hierarchical clustering and K-Means clustering to find the groups based on their mean values.

Protein sequence search algorithms are powerful tools in bioinformatics. They are used to identify the functional relationship, defining subgroups of large protein families and to establish the structural and functional relationship at the molecular level. The protein structure and function prediction was done by using the tool Interpro-scan. Inter-proscan (http://www.ebi.ac.uk/Tools/pfa/iprscan/) is a publicly available tool to predict and scan the domains of the protein queried by feeding the amino acid sequences. The 3D structure of the protein was generated and compared with other proteins by using Swiss modeller and viewer (http://swissmodel.expasy.org/?pid=smh01).

For the construction of phylogenetic trees ClustalX2 was used. ClustalX2 (http://www.clustal.org/ and http://www.clustal.org/clustal2/ Release Version 2.1 2010-11-17) a general purpose open source Multiple Sequence Alignment (MSA) program that uses GUI interface for the input of DNA or protein sequences (Larkin *et al*., 2007; Chenna *et al*, 2003). Once the MSA was obtained the next step was to convert the alignment into the score matrix where the percent identity, similarity and e-values were obtained. The expected values represented the sequence similarities between the target and query sequence. The phylogenetic tree was calculated and was generated using the tree view software. The trees were based on the Un- paired Group Mean Method (UPGMA) or the most widely used Neighbour Joining Tree (NJ). The trees were bootstrapped (1000 replicates). Other softwares like MEGA (Kumar *et al.,* 2008) and Jalview (Waterhouse *et al*., 2009) were also used for the alignment and construction of the phylogenetic trees. The flow chart of *in silico* analysis of Rab GTPase is shown in chapter 3.

The consensus logo for the conserved motifs were generated and printed by using the Jalview software package.

The tomato genome was published in Nature on May 31, 2012 (The Tomato Genome Consortium 2012 *Nature* 485: 635-641).The official annotation for the tomato genome is provided by the International Tomato Annotation Group (ITAG) a multinational consortium, funded in part by the EU-SOL project. Pre-release ITAG VERSION 2.40 of the assembled draft genome sequence of *Solanum lycopersicum* cv. Heinz 1706 is now available for researcher.

The Solyc IDs of tomato Rab GTPase were identified by following the same pathway of analysis as was utilized in TIGR tomato gene index database from the latest tomato whole genome sequence available at SGN database (http://solgenomics.net/) with the current release ITAG version 2.4.

in silico analysis of other proteins involved in membrane trafficking was carried out using the same pipeline and pathway of analysis as described for Rab GTPases.

3. *in silico* analysis of Small GTPases and other proteins involved in intracellular vesicle trafficking

3.1 Introduction, history and nomenclature of Small GTPases

The small GTPases belong to a very large super family belonging to Ras GTPases. GTPase stands for gunosine triphosphate. The Ras GTPase derives its name from the Rat Sarcoma virus. Ras super family largely consists of RAS, RAL, RAB, RHO, SAR, ARF and RAN GTPase (Wennerberg *et al.,* 2005). The Ras homologous GTPase are called Rho GTPase, which were first discovered in snails. The Rho GTPases are also found in yeast, drosophila, rat and humans. In case of plants the Rho orthologues in plants are termed as Rho of Plants (ROP GTPase). Ral GTPase derives its name from Ras-like GTPase. Touchot *et al.,* in 1987 isolated a mammalian ortholog of Ypt1 from a rat brain cDNA library; hence the Ras like GTPase from the rat brain was called Rab GTPase. SAR GTPase stands for secretion associated and Ras related GTPase which was first identified in *S. cervisiae.* ADP ribosylation factor (ARF) was first found in cholera toxin catalysed by ARF GAP of adenylate cyclase. The GTPase involved in nucleo-cytoplasmic transport was named as Ras-related nuclear protein commonly known as RAN GTPase (Cox and Der, 2010).

Ras GTPases are involved in various signalling pathways resulting in the transcription, cellular differentiation and proliferation. Rab and Arf GTPases are involved in vesicle associated processes including the formation or recruitment of other members for trafficking. Rho GTPases are involved in the regulation of cell shape, cytoskeleton and cell migration and cell polarity. Ran GTPases are involved in the nuclear import and exports, regulation of the nuclear envelop as well as control of formation of the spindle fibres (Bos *et al*., 2007).

Almost 17 percent of the gene products enter the endomembrane system for the transport of different proteins (Rutherford and Moore, 2002). Members of the Rab family are critical for the proper movement of the transport vesicles between different compartments in the eukaryotic cells (Zerial and Stenmark, 1993)

Most highly conserved residues of Rab GTPases are involved in nucleotide binding and hydrolysis which is common in all Ras like GTPase. The effecter region interacts with the subclass specific GTPase activating protein (GAP) (Zerial, 1992).

3.2 Rab GTPase

The Rab GTPase was first identified in *S. cervisiae* which was involved in regulation of vesicle trafficking (Gallwitz *et al*., 1983). The mammalian ortholog of Ypt1 from a cDNA library of rat brain was isolated by Touchot *et al.* (1987).

The Rab family of protein is a member of the Ras super family of monomeric G proteins called small GTPases. Rab GTPases (Ras-related in brain) are the key players in vesicle trafficking (Touchot *et al*., 1987). Rab GTPases have been found in several organisms including *Saccharomyces cervisiae* (11 members), *Cenorhabditis elegans* (29 members), *Drosophila melanogaster* (26 members) (Bock *et a*l., 2001), and *Arabidopsis thaliana* (57 members).

3.3 Rab GTPase cycle

The Rab GTPase cycles between an active GTP bound state and inactive GDP bound state. The hydrolysis of the GTP to GDP by releasing the phosphate is catalysed by GTPase activator protein (GAP). After the hydrolysis the GDP bind to the GDP dissociation inhibitor (GDI). This GDI masks the geranylgeranyl moiety at the C terminal domain of the Rab GTPase which is essential for the membrane association and its availability in cytosol. The guanine nucleotide exchange factor (GEF) activates the Rab and catalyses the conversion of inactive Rab GDP to active Rab GTP. To complete the signalling process for attachment with the organelle membrane Rab GTPase detaches from Rab GDI and travels towards the target membrane facilitated by the GDI displacement factor (GDF) (Dirac-svejtrup *et al.*, 1997).

3.4 Different Classes of Rab GTPases

In *Arabidopsis*, the 57 Rab GTPases have close homology to only eight of the types found in mammals. The plant Rab family consists of eight subfamilies, designated AtRabA–AtRabH, each with counterparts in yeast or animals. These are further divide into 18 subgroups, most of which are plant-specific and of unknown function (Pereira-Leal and Seabra, 2001; Rutherford and Moore, 2002; Vernoud *et al*., 2003; Deneka *et al*., 2003). The few experimental data available to describe Rab GTPases are mainly restricted to the RabA, RabF and RabG subfamilies.

Most notably, the two mammalian Rab11 GTPases correspond to the *Arabidopsis* RabA clade, Rab11/RabA has 26 members out of 57 Arabidopsis Rabs, divided into six sub groups (RabA1 – RabA6). RabA1 have been implicated in the transport between TGN and PM and were shown to be important to confer salinity tolerance (Asaoka *et al*., 2013). The RabA2 subgroup is closely related to human Rab11 and Rab25, which are generally thought to be associated with the polarised recycling of proteins at the PM. This has led to the hypothesis that these sub groups may have evolved different functions (Rutherford and Moore, 2002).

RabB (Rab2) and RabD (Rab1) are expected to be involved in ER to Golgi transport in plants (Batoko *et al*., 2000; Cheung *et al*., 2002). RabC (Rab18) and RabF (corresponding to mammalian Rab5 and/or Rab22), and RabG (Rab7) proteins are expected to be associated with endocytosis. RabE is related to the Golgi to PM associated Rabs of mammals such as Rab8 and seems to have the same role in plants (Zheng *et al*., 2005). RabFs may be involved in either endocytosis or vacuolar trafficking (Ueda *et al*., 2001; Grebe *et al*., 2003; Sohne *et al*., 2003; Bolte *et al*., 2004; Kotzer *et al*., 2004; Lee *et al*., 2004). RabH (Rab6) complements its yeast homologue, *Ypt6*, which is implicated in retrograde Golgi to ER transport (Bednarek *et al*., 1994).

RabA/Rab11 group is diversified in case of plants to fulfil different functions in different tissues of the plants. RabA clade is subdivided into six different structural subclasses RabA1 to RabA6 which is homologous to Rab11 and Rab25 in mammals. RabA3 and RabA4 are involved in the transport from Golgi to the endosomes and to the plasma membrane. In case of AtRabA5c which is involved in the transport from the Golgi to the trans-Golgi network (TGN) in pollen tubes. RabA interacts between the various endomembranes via the actin cyto-skeleton. The organelle motility is primarily based on the actin dependent movement in plants. In case of animals the microtubules play major role in the movement of the vesicles to different endomembranes. RabA2 and RabA3 have been shown to contribute to the trafficking to cell plate formation in plants (Chow *et al*., 2008). RAB-A2 and RAB-A3 GTPases were localized to membranes that specifically label the margins of growing phragmoplasts. RabA3 which is found in Arabidopsis effectively integrates the light and brassinosteroid signalling pathway in response to etiolation in case of tobacco.

RabA1c has been co-localized with RabA2 and RabA4 at TGN and is relocated to cell plate during cytokinesis. The members of Rab-A1 proteins have been shown to be involved in auxin-mediated responses (AUX1, PIN2) in root growth and mutations in TRAPPII (which functions as GEF for Rab11), a protein complex that acts upstream of RAB-A1C (Qi and Zheng, 2012).

RabA4 and anti-phosphatidylinositol-4 kinase (PI-4K) antibodies have been reported to bind to the Golgi-associated and free TGN compartments but not trans-Golgi cisternae. RabA4b and PI-4Kβ1 localize to budding secretory vesicles in the TGN (Kang *et al*., 2011).

The role of cell wall metabolism and characteristic functions and different roles of Rab GTPase in plants have been extensively reviewed by Lycett (2008). The mammalian Rab11 GTPases are associated mainly with endocytosis and recycling endosomes (Zerial and McBride, 2001) but evidence from plants suggested that some groups are associated with transport to the cell membrane (Ueda *et al*., 1996; Lu *et al*., 2001; Inaba *et al*., 2002; de Graaf *et al*., 2005).

Elements of the secretory pathway, such as Rab GTPases, have been shown to be expressed in a ripening related manner in fruit (Loraine *et al*., 1996; Zainal *et al*., 1996; Zegzouti *et al*., 1999; Lu *et al*., 2001; Park *et al*., 2006). Three Rab1 proteins from tomato are expressed strongly in fruit (Loraine *et al*., 1996). One of these (*LeRab1C*) is expressed most strongly in expanding green fruit and the other two (*LeRab1A* and *LeRab1B)* more strongly in ripe fruit. The corresponding proteins differed in their ability to interact with geranylgeranyl transferases (GGTase) from yeast, mammalian cells and plant cells. Thus, though they are all the same class of protein, presumably regulating the same step in the secretory pathway and they are all expressed in fruit, there may be subtle differences in their roles. *Rab8* and *Rab11* genes have also been found to be preferentially expressed in tomato fruit (Zegzouti *et al*., 1999; Lu *et al*., 2001).

It has been suggested that the Rab GTPases regulate secretion of the enzymes that modify the cell wall to bring about fruit softening (Loraine *et al*., 1996; Zainal *et al*., 1996). This has been investigated for the Rab11 class, first identified in ethene-treated mango fruit mesocarp (Zainal *et al*., 1996). A tomato orthologue (*LeRab11a*) was silenced by transformation with antisense constructs. The transgenic tomato

plants had fruit that remained firm for a very long period and it was shown that the levels of extracted polygalacturonase and pectinesterase were reduced in these fruits (Lu *et al*., 2001).

The *Rab11* antisense tomato plants also showed distinct physiological and developmental differences, including altered floral organ identity, highly branched inflorescences, rumpled leaves, ectopic shoots from leaves, determinate growth, reduced apical dominance and altered ethene production. These effects are reminiscent of developmental abnormalities and altered hormone levels, where Rab11 GTPases have been over expressed (Kamada *et al*., 1992; Sano *et al*., 1994; Aspuria *et al*., 1995) and are not likely to be due to altered trafficking to the cell wall.

The gene *LeRab11a* has been shown to be expressed much more strongly in expanding green fruit than in ripe fruit and the reduction in pectinesterase and β-galactosidase levels was much greater in green fruit. In a study using tobacco leaf protoplasts, LeRab11a-GFP fusions were shown to be associated with the *trans*-Golgi network. Dominant negative mutant versions of the *Rab* gene also inhibited trafficking of secreted GFP. Furthermore, inhibiting the SYP121 pathway as well as Rab11a caused a greater inhibition of trafficking than just the *LeRab11a* dominant negative mutant alone, whereas inhibition of SYP122 did not. This indicates that SYP122 and Rab11a are probably involved in the same pathway but SYP121, which has been implicated in ABA responsive secretion may be involved in a different pathway (Leyman *et al*., 2000).

However, several Rab GTPases have been shown to be expressed in a range of actively growing or highly secretory tissues (Cheung *et al*., 2002; Lu *et al*., 2001) and inhibition of LeRab11a did not inhibit overall growth (Lu *et al*., 2001). It is also possible that different proteins and polysaccharides destined for the cell wall are delivered by different vesicles and support for this has recently emerged (Leucci *et al*., 2007).

Table 3.1 shows the list of Rab11/RabA GTPase obtained and confirmed from the TAIR database with their AGI codes and sequences for using it as reference for searching the tomato Rab GTPases in the TIGR, SGN and TED databases.

Table 3.1 List of the Arabidopsis RabA/Rab 11 GTPase from TAIR database

Group	Ian Moore's nomenclature	Zhang nomenclature	Gene name/ AGI Code
At Rab A1	*AtRABA1a*	AtRab11D2	At1g06400
	AtRABA1b	AtRab11D1	At1g16920
	AtRABA1c	AtRab11D4	At5g45750
	AtRABA1d	AtRab11D3	At4g18800
	AtRABA1e	AtRab11C1	At4g18430
	AtRABA1f	AtRab11C4	At5g60860
	AtRABA1g	AtRab11C5	At3g15060
	AtRABA1h	AtRab11C3	At2g33870
	AtRABA1i	AtRab11C2	At1g28550
At Rab A2	*AtRABA2a*	AtRab11A1	At1g09630
	AtRABA2b	AtRab11B3	At1g07410
	AtRABA2c	AtRab11B2	At3g46830
	AtRABA2d	AtRab11B1	At5g59150
At Rab A3	*AtRABA3*	AtRab11F1	At1g01200
At Rab A4	*AtRABA4a*	AtRab11E1	At5g65270
	AtRABA4b	AtRab11E2	At4g39990
	AtRABA4c	AtRab11E5	At5g47960
	AtRABA4d	AtRab11E4	At3g12160
	AtRABA4e	AtRab11E3	At2g22390
At Rab A5	*AtRABA5a*	AtRab11G1	At5g47520
	AtRABA5b	AtRab11G2	At3g07410
	AtRABA5c	AtRab11G3	At2g43130
	AtRABA5d	AtRab11G4	At2g31680
	AtRABA5e	AtRab11G5	At1g05810
At Rab A6	*AtRABA6a*	AtRab11H1	At1g73640
	AtRABA6b	AtRab11H2	At1g18200

Format of chromosomal based nomenclature used in the table (According to TAIR):
AT (*Arabidopsis thaliana*); 1,2,3,4,5 (chromosome number); G (gene) followed by five-digit code, numbered from top/north to bottom/south of chromosome)

RabB/Rab2 is not found in yeast but in case of mammals it is localized in the cis-Golgi membrane interacting with the different proteins in Golgi matrix. Mutation in Rab2 results in the clustering of various vesicular tubular structures between ER and Golgi. The dominant negative mutants of Rab2 results in slowing down of the growth of pollen tubes because of the inhibition of the ER to Golgi transport. Table 3.2 shows the AGI codes obtained and their nomenclature for RabB/2 GTPase

Table 3.2 Arabidopsis Rab2/RabB from TAIR database

Ian Moore's nomenclature	Zhang nomenclature	Gene name
AtRABB1a	AtRab2A3	At4g17160
AtRABB1b	AtRab2A2	At4g35860
AtRABB1c	AtRab2A1	At4g17170

RabC/Rab18 is involved in the endocytic transport and in case of mammals is involved in the polarization of the epithelial cells. There are three Rab18/RabC in Arabidopsis, classified into 2 groups. One with single member C1 and other with 2 members C2a and C2b. Table 3.3 shows the AGI codes obtained and their nomenclature for RabC/18 GTPase.

Table 3.3 Arabidopsis Rab 18/Rab C from TAIR database

Ian Moore's nomenclature	Zhang nomenclature	Gene name
AtRABC1	AtRab18A1	At1g43890
AtRABC2a	AtRab18B1	At5g03530
AtRABC2b	AtRab18B2	At3g09910

RabD/Rab1 is divided into two subgroups RabD1 and RabD2. Rab D1 is highly conserved in both the monocots and dicots. The dominant negative mutants of RabD2 arrest the transport from ER to Golgi which is required for the normal anterograde trafficking from ER to Golgi (Pinheiro *et al*., 2009). Table 3.4 shows the AGI codes obtained and their nomenclature for RabD/1 GTPase.

Table 3.4 Arabidopsis Rab1/Rab D from TAIR database

Ian Moore's nomenclature	Zhang nomenclature	Gene name
AtRABD1	AtRab1A1	At3g11730
AtRABD2a	AtRab1C1	At1g02130
AtRABD2b	AtRab1B1	At5g47200
AtRABD2c	AtRab1B2	At4g17530

RabE/Rab8 is homologous to Rab8 and Rab10 in mammals, Sec4 in *S. cervisiae* and Ypt 2 of *S. pombe.* RabE is involved in the post Golgi to plasma membrane transport. Table 3.5 shows the AGI codes obtained and their nomenclature for RabE/Rab8 GTPase.

Table 3.5 Arabidopsis RabE/Rab8 from TAIR database

Ian Moore's nomenclature	Zhang nomenclature	Gene name
AtRABE1a	AtRab8A1	At3g53610
AtRABE1b	AtRab8A2	At5g59840
AtRABE1c	AtRab8A3	At3g46060
AtRABE1d	AtRab8B1	At5g03520
AtRABE1e	AtRab8B2	At3g09900

RabF/Rab5 is homologous to Rab5 and Rab22 in mammals. RabF is involved in the endocytic sorting pathway. RabF associates to the membrane via the amino terminal N-myristoylation and palmitoylation rather than by the carboxy terminal domain. RabF1 was first identified in lotus and also found to be important in case of halophytes for the regulation of salt stress. The dye FM64 is generally used as the endocytic marker for the localization of RabF1 and RabF2. Table 3.6 shows the AGI codes obtained and their nomenclature for RabF/Rab5 GTPase.

Table 3.6 Arabidopsis RabF/Rab5 from **TAIR database**

Ian Moore's nomenclature	Zhang nomenclature	Gene name
AtRABF1	AtRab5A1	At3g54840
AtRABF2a	AtRab5B1	At5g45130
AtRABF2b	AtRab5B2	At4g19640

RabG/Rab7 is involved in the transport of the vesicles to the vacuoles and lysosomes. Ypt7 in yeast undergo homotypic fusion with the target membrane. The antisense transgenic line of RabG3 in soybean interferes with the nodule formation in the roots. Table 3.7 shows the AGI codes obtained and their nomenclature for RabG/Rab7 GTPase.

Table 3.7 Arabidopsis RabG/Rab7 from **TAIR database**

Ian Moore's nomenclature	Zhang nomenclature	Gene name
AtRABG1	AtRab7C1	At5g39620
AtRABG2	AtRab7A3	At2g21880
AtRABG3a	AtRab7A2	At4g09720
AtRABG3b	AtRab7A1	At1g22740
AtRABG3c	AtRab7B2	At3g16100
AtRABG3d	AtRab7B1	At1g52280
AtRABG3e	AtRab7B4	At1g49300
AtRABG3f	AtRab7B3	At3g18820

RabH/Rab6 in case of Arabidopsis is divided into five sub classes. Rab6A is involved in the retrograde transport via the Golgi stacks and it role is also implicated for the slow movement of the COP-I independent retrograde transport from Golgi to ER. The splice variants of Rab6A interfere with the retrograde transport from endosomes to Golgi. Table 3.8 shows the AGI codes obtained and their nomenclature for RabH/Rab6 GTPase.

Table 3.8 Arabidopsis RabH/Rab6 from TAIR database

Ian Moore's nomenclature	Zhang nomenclature	Gene name
AtRABH1a	AtRab6A1	At5g64990
AtRABH1b	AtRab6A3	At2g44610
AtRABH1c	AtRab6A2	At4g39890
AtRABH1d	AtRab6A4	At2g22290
AtRABH1e	AtRab6A5	At5g10260

3.5 Introduction to *in silico* analysis

The analysis of the publicly available database offers a cost effective and rapid approach to elucidate the genome and transcriptome of an organism. The expressed sequence tags (EST) and their consecutive assembly results in the optimization of the Tentative Conesus (TC) sequence information. The EST represents the short, unedited and randomly selected single pass reads of the sequences derived from the cDNA libraries which provide an alternative to whole genome sequencing. This analysis enables gene discovery, complete genome annotation, aids in identification of the structure of gene and proteins as well as in validating the alternate transcripts.

Protein sequence search algorithms are powerful tools in bioinformatics. They are used to identify the functional relationship, defining subgroups of large protein families and to establish the structural and functional relationship at the molecular level. *In silico* method offer valuable tools to examine the hypothesis and also helps to facilitate and narrow down the range of experimental options. The first step is data mining, which relates to mining of publicly available databases for finding the sequence information to generate a customized database of all DNA/Protein sequences of interest. The pattern based searching of the database (PSI-BLAST) is useful tool to search sequences of interest (Altschul *et al.,* 1990). ClustalX2 is a general purpose open source multiple sequence alignment (MSA) program that uses GUI interface for the input of DNA or protein sequences (Thompson *et al.,* 1997). Once the MSA is obtained the next step is to convert the alignment into the score matrix where the percent identity, similarity and expected values represent the sequence similarity between the target and query sequence. The phylogenetic tree is calculated and viewed using the tree view software or other software e.g. MEGA for generating the alignment and construction of the phylogenetic trees (Kumar *et al.,* 2008)

3.6 *In silico* analysis of Rab GTPase

The objective of the *in silico* analysis was to find all the available Rab genes in tomato. In order to find Rab genes in tomato TAIR database was screened to find all the small GTPase and their sequences, which was then followed by the search of Rab genes in the DFCI tomato gene index of TIGR database to extract the gene sequence of available *Rab* genes.

The information about each *Rab* gene was extracted from the TGI database including the sequences. After the Blast search, the nearest homologues were found and were enlisted in a table for screening the sequences manually. The table for comparison of Tomato Rab GTPase with TC sequences (Nucleotide sequences), their coding sequences (CDS) and complete ORF's (Amino Acid sequences) was generated. Expression profiles for all the tentative consensus (TC) numbers of Rab genes were extracted from TIGR DFCI Tomato Gene index.

The available nucleotide and protein sequence were blasted into the SGN Tomato database to search for the corresponding and homologous sequence having the perfect to nearest homology of sequences available. As a result Rab GTPase homologs and their corresponding SGN-Unigene Ids were extracted.

Following the screening of Blast results of all individual Rab GTPases with individual TC Ids from TIGR DFCI TGI the best hits having the highest score and good E-value cut-off were arranged in a database to compare their sequences. The particular emphasis was given to the genes that were expressed in tomato in a ripening related manner or are induced at ripening, during tomato flowering, tomato fruit development and the genes that were repressed during ripening. A list comparing the ripening induced versus repressed genes was generated.

The data regarding different tissue specific gene expression was also extracted from the database. Fruit, pollen, root, flower, callus, leaf, ripening, shoot and seed specific genes were also screened.

The specific TC for *Rab* genes from TIGR and the corresponding SGN unigene ids were used to extract the Probe Ids from the Tomato Expression Database (TED). The list of probe ids specific to Rab was used to screen the expression profiles of the different Rab genes at different developmental stages of tomato fruit ripening. The TED Database and the TED Data warehouse were searched to obtain the raw data of available micro-array experiments. The results of the expression profiling were used to generate the cluster diagram for generating expression pattern of Rab genes at different developmental stages.

The flowchart as shown in fig. 3.1 shows the schematic diagram of the *in silico* profiling and database searching strategy to find Rab GTPases in Tomato.

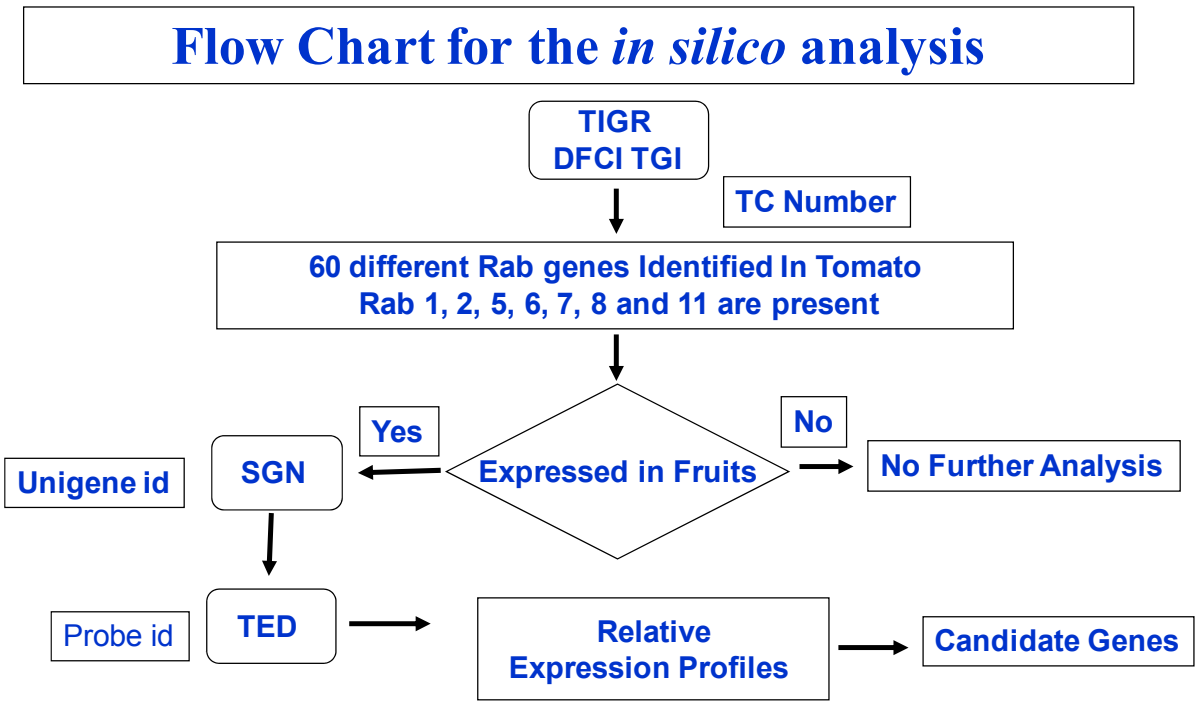


Fig 3.1 The flow chart of the *in silico* analysis method used for retrieving the Rab GTPases in tomato. The reference protein sequences of Arabidopsis Rab GTPases were retrieved from the TAIR database and were used as query for BLAST in TIGR DFCI TGI database. The e-values, score and sequence were inspected and those found fitting the criteria were used for generating MSA and phyolgenetics trees for classification. After the confirmation, the same approach was used to screen the SGN database for unigenes and for extracting the Probe ids from TED database. The Rab GTPases were screened for their expression in the fruit tissues in order to hunt for the candidate genes.

Following the above approach for screening the databases from TIGR DFCI Tomato gene index a total of 60 *Rab* genes were found. The individual sequences and the protein sequences were carefully analysed and checked whether they actually belong to the Rab GTPase family. The sequences with incomplete length or half length and missing the important Rab Specific and Rab sub family specific motifs were not considered.

Table 3.9 Rab genes identified by the *in silico* analysis from the TGI database

Class of Rab	Total Genes	Role
Rab 1/RabD	9	ER →Golgi
Rab 2/RabB	4	ER→ Golgi
Rab 5/RabF	5	PVC→Vacuole
Rab 6/RabH	5	ER →Golgi→ TGN
Rab 7/RabG	7	PM→Vacuole
Rab 8/RabE	8	Golgi → PM
Rab 11/RabA	19	TGN→PM
Rab 18/RabC	3	Early Endosome
Total	60	

3.7 Protein structure and function prediction of Rab11a/RabA1a GTPase

The protein sequence of the LeRab11a/RabA1a, (Q9SCB1) was used to analyze the protein sequence by the inter-pro scan in order to identify the family and sub family specific domains. The amino acid sequence of Le Rab11a was fed into the inter-pro scan server to obtain the domain scans of tomato Rab. The results from the inter-pro scan shows all the GTP binding domains and the protein belongs to the Ras superfmaily of GTPase as shown in the fig 3.2. IPR003579 Id is for the Ras small GTPase of Rab type.

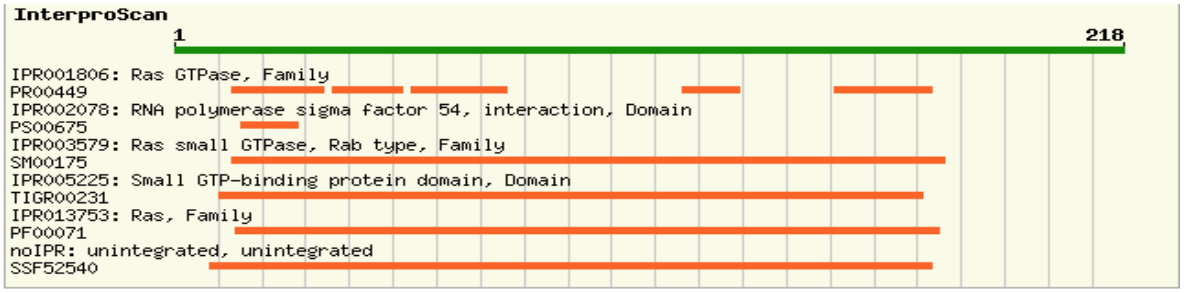


Fig 3.2 Inter-pro Scan of LeRab11a/LeRabA1a GTPase

The inter-pro scan of the tomato Rab11a shows that it belongs to RAS superfamily and contains small GTP binding domains.

Secondary structure prediction for the LeRab11 GTPase (Q9SCB1) was done using the Muster program. Fig. 3.3 below shows the helix and loops specific and conserved in Rab GTPases. C represents the coil, H represents the helix and E represents the Sheets which are conserved in most of the Rab GTPases.

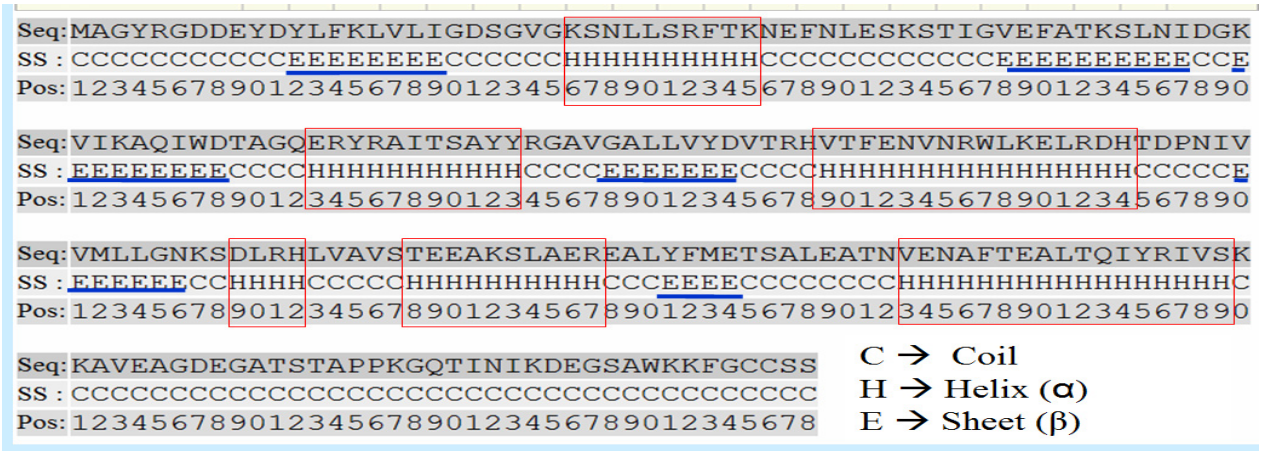


Fig 3.3 Secondary structure of LeRab 11 GTPase.

The secondary structure of LeRAB11a is shown. The amino acid sequence of the protein is aligned with the features of secondary structure. Where C represents coiled coil, H represents the Helix (Red boxes) and E represents the beta sheets (Blue underline). The numbers from 1 to 9 represent the position and turns of the protein structure.

The protein sequence of tomato Rab11a/LeRabA1a was queried in the Protein Homology/analogY Recognition Engine V 2.0 (Phyre2 server current version). The fig. 3.4 shows results of the analysis for secondary structure, domain analysis, the characheteristic G motifs, switches, Rab family (RabF) and Rab Sub-Family (Rab-SF) specific motifs. The Phyre2 analysis of the tomato Rab 11 GTPase highlighted the following characteristic features:

Feature 1: GTP/Mg^{2+} binding site

Feature 2: putative GEF interaction site [K--GVEFATKS] [V-K] [polypeptide binding site]

Feature 3: putative GDI interaction site [IG-E] [WD] [Y-A-TIA] [polypeptide binding site]

Feature 4: putative Effector interaction site [IVEF] [Q-W] [RY-I-RGAV] [I]

Feature 5: Switch-I region [F-KSTIGVEFA] One of two surface loops that undergo conformational changes upon GTP binding, also called effector region.

Feature 6: Switch-II region [GERYRAITIAYY] One of two surface loops that udergo conformational changes upon GTP binding.

Feature 7: G1 box- G1 box motif: GXXXXGK [T/S]; signature motif of phosphate-binding loop. G1 box is also known as the P-loop and the Walker A motif

Feature 8: G2 box- G2 box motif: [T] only 'Thr' is conserved throughout the superfamily, but surrounding residues are conserved within families. G2 overlaps with the Switch I region, also known as the effector region.

Feature 9: G3 box

- G3 box motif: DXXG, G3 box overlaps the Switch II region, which includes the Walker B
- 'Asp'- forms a water-bridged contact with Mg^{2+}
- 'Gly' is hydrogen bonded to the gamma phosphate of GTP through the backbone amide.

Feature 10: G4 box G4 box motif: [N/T] KXD

Feature 11: G5 box G5 box motif: [C/S] A [K/L/T]

Feature 12: Rab family motif 1 (RabF1) [IGVEF] Rab family motif region 1: A region with high homology within the Rab family.

Feature 13: Rab family motif 2 (RabF2) [KAQIW] Rab family motif region 2: A region with high homology within the Rab family

Feature 14: Rab family motif 3 (RabF3) [RYRAIT] Rab family motif region 3: A region with high homology within the Rab family.

Feature 15: Rab family motif 4 (RabF4) [YYRGA] Rab family motif region 4: A region with high homology within the Rab family.

Feature 16: Rab family motif 5 (RabF5) [LVYDVT] Rab family motif region 5: A region with high homology within the Rab family

Feature 17: Subfamily motif 1 (RabSF1) [DYLFK] Rab subfamily motif region 1: A region with high homology within a Rab subfamily.

Feature 18: Rab Subfamily motif 2 (RabSF2) [NLLSRFTKNEFN-ESKS] Rab subfamily motif region 2: A region with high homology within a Rab subfamily.

Feature 19: Rab Subfamily motif 3 (RabSF3) [IVVML] Rab subfamily motif region 3: A region with high homology within a Rab subfamily.

Feature 20: Rab Subfamily motif 4 (RabSF4) [TQIY] Rab subfamily motif region 4: A region with high homology within a Rab subfamily.

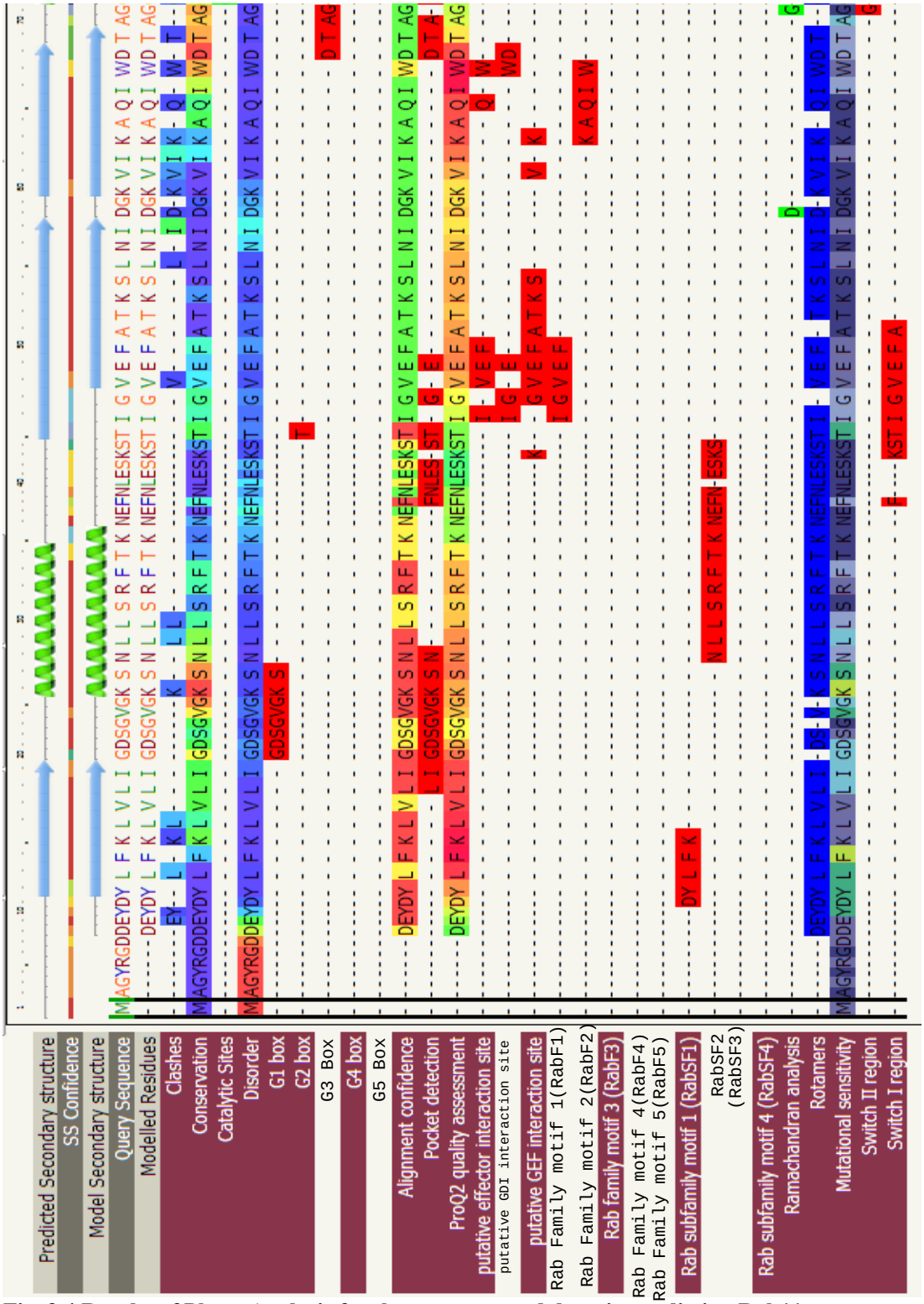


Fig. 3.4 Results of Phyre Analysis for the structure and domain prediction Rab11 GTPase. The protein sequence of tomato Rab11 was queried in Phypre server. The fig. shows secondary structure, the characheteristic G motifs, switches, Rab family (RabF) and Rab Sub-Family (Rab-SF) specific motifs.

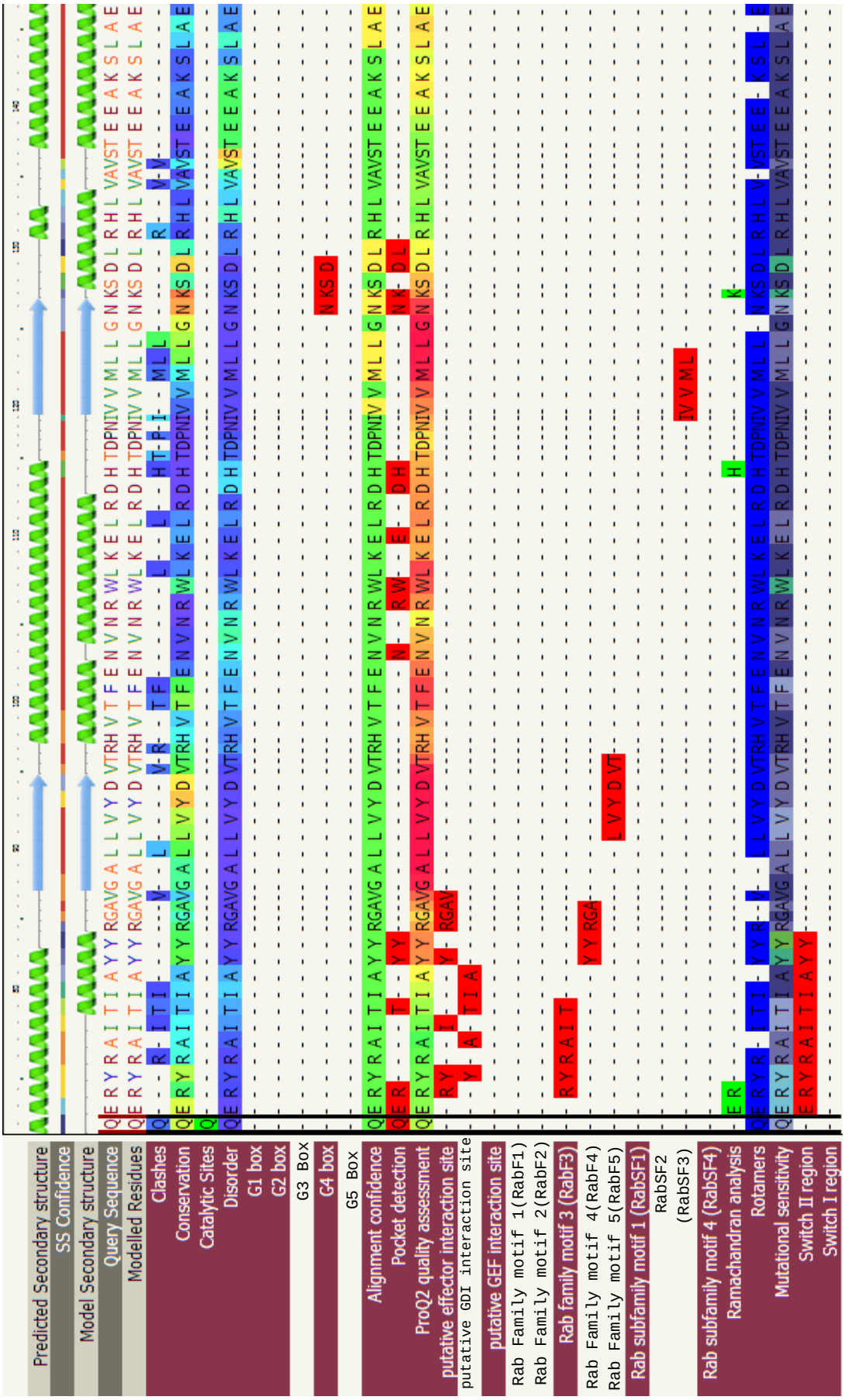


Fig. 3.4 Results of Phyre Analysis for the structure and domain prediction of LeRab11 GTPase cont...

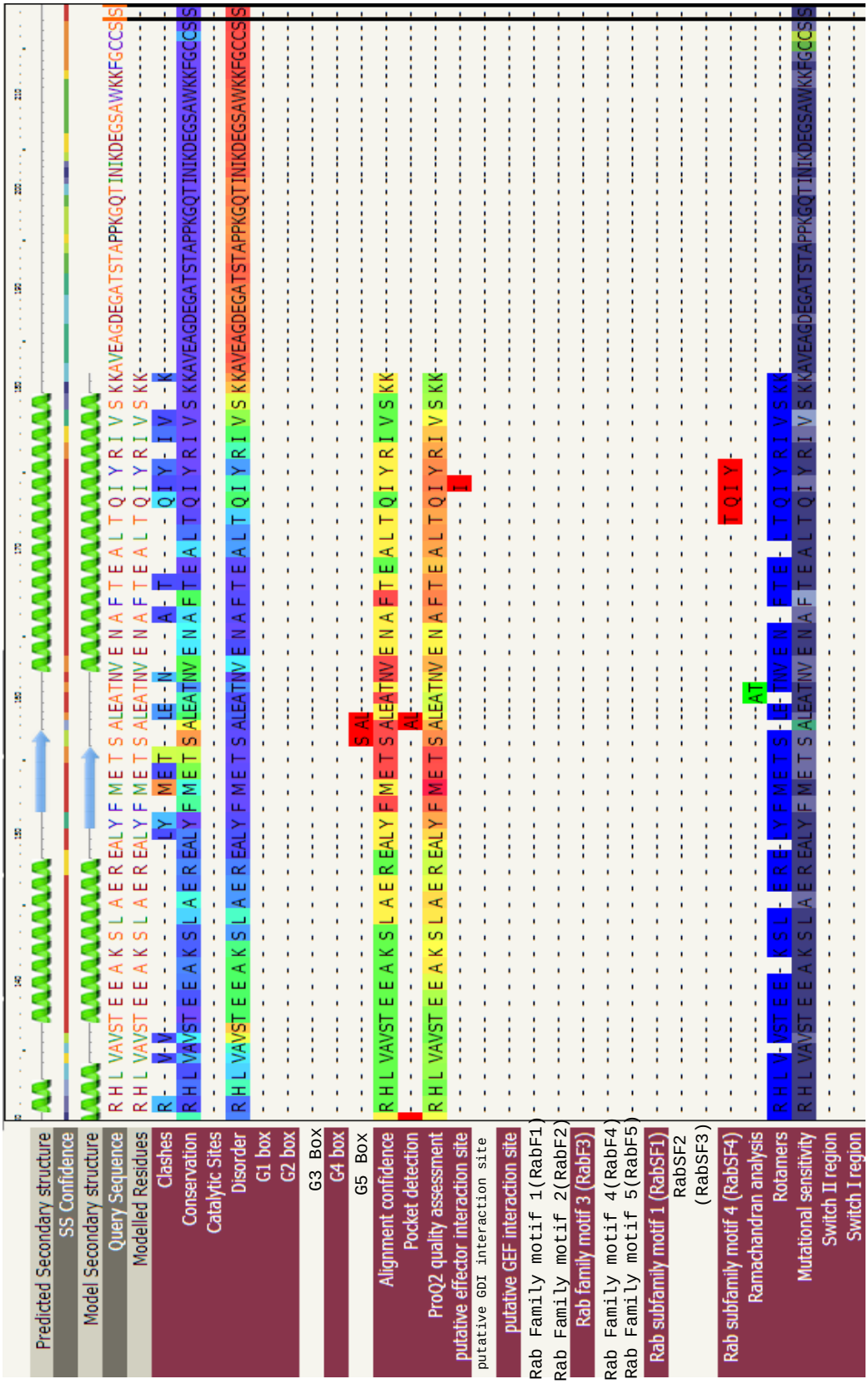
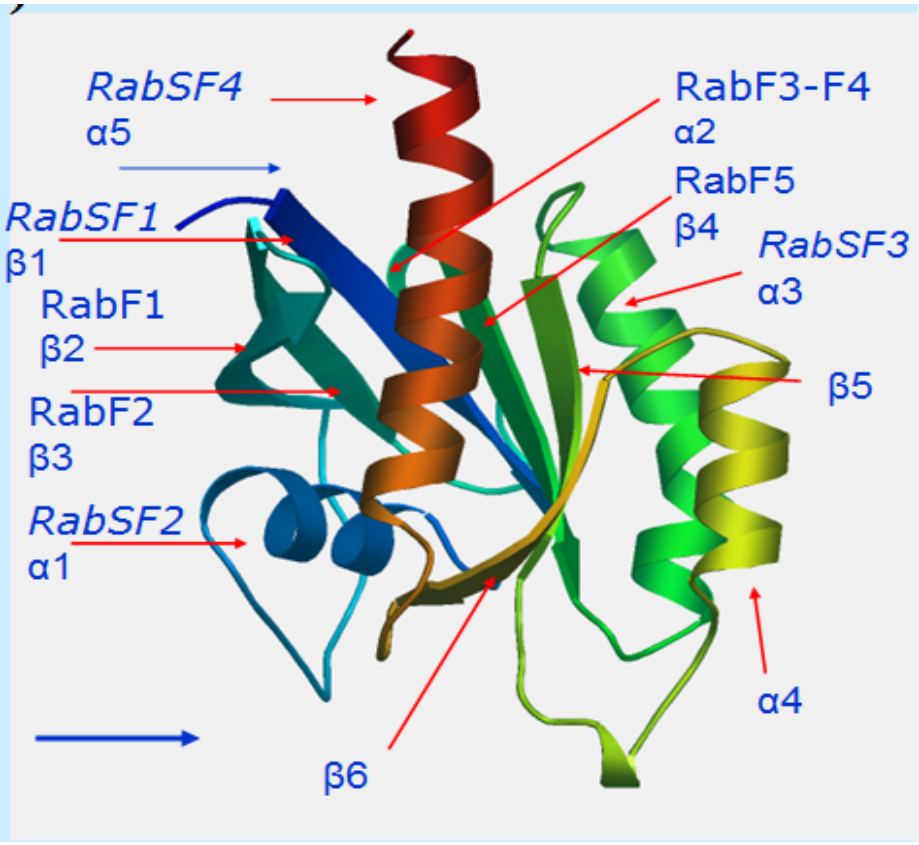


Fig. 3.4 Results of Phyre Analysis for the structure and domain prediction of LeRab11 GTPase cont...

Rab GTPase consists of Rab Specific motifs (RabF motifs) containing the PM and G motifs which is clustered around the switch regions. The specificity to bind to the Rab interacting partners is governed by the switch regions (Merithew *et al.,* 2001).The G stands for the 'Guanine interacting motif' which ranges from G1-G3 and PM stands for phosphate interacting with magnesium MG^{2+} containing PM1-PM3 motif (Valencia, 1991). The GTP binding motifs G1 (P-Loop) coordinates the phosphate interaction, G2 motif (Switch-I) in which threonine brings about the catalysis, G3 motif (Switch-II) contains glycine which forms the hydrogen bond with the γ-phosphate of GTPase (Praefcke and McMahon, 2004).



Model dimensions (Å): **X**:48.248 **Y**:51.208 **Z**:41.241

Fig 3.5 The 3D Structure of Le Rab11a GTPase

The 3-dimensional structure of *Le Rab11a* in form of Ribbon diagram is shown. Different colours show the different Rab family specific and sub family specific beta-sheets (Bold Arrows), alpha-helix (spring like helix) and coils (wire loops).
Image coloured by rainbow N → C terminus.

As shown in fig.3.5 the 3D structure was generated for LeRab11 protein sequence using the reference of '2f9ma' from the swiss modeller and arranged using the free open source Ribbon software. Image was coloured by rainbow N → C terminus. The ribbon model's dimensions are **X**: 48.248 **Y**: 51.208 **Z**: 41.241 in Å. The Rab11 GTPase ribbon model in the figure was modelled with 100.0% confidence by the single highest scoring template with reference to '2f9ma'**.** All the characteristic Rab family motifs and Rab Subfamily motifs are labelled by arrows.

The specificity determining regions are called Rab sub-family specific (RabSF) motifs (Pereira and Seabra, 2000). The Rab family motif has 5 motifs from RabF1 to RabF5. RabF1 contains "IGVDF" which is Loop 2 (β_2), Rab F2 contains "KLQIW" (β_3), RabF3 consists of "RFRSIT" which is Loop4 (α_2), RabF4 contains "YRGA" loop5 (α_2) and Rab5 contains "LVYDIT" loop-6 (β_4). Rab F2 is clustered between the sheets β_3 and β_4. In case of Rab subfamily specific sequence motifs (RabSF) RabSF1 is β_1, Rab SF2 is (α_1) Loop-2 which regulates GEF and GAP, RabSF3 is loop 7 (α_3) which acts as effector's domain and RabSF4 is α_5 which is located in the hyper-variable C-terminal domain of the Rab11 GTPase which determines the localization of a particular Rab (Pereira and Seabra, 2000).

3.8 Multiple Sequence Alignment and Phylogenetic analysis of Rab GTPases in Tomato.

Exhaustive blast searches were carried out in the TIGR DFCI TGI database (following the Release11, 12 and the last Release13). The results were carefully analysed by comparing the E-values, score, hits, percent identity, similarity and visual inspection of the possible ORFs and amino acid sequence of the proteins. The incomplete ORF were culled from the analysis. In order to classify Rab GTPases in tomato the amino acid sequence of each Arabidopsis Rab protein was used for the multiple sequence alignment (MSA). The MSA were performed by using the basic ClustalX2, MEGA, Bioedit and Jalview programs with default settings. The results of MSA of tomato Rab GTPases are shown in Fig.3.6 (Fig.3.6.1 to Fig.3.6.7) for Rab1 to Rab11 GTPase according to the release 12 of TIGR TGI. The conservation, consensus and the Consensus logo were generated by Jalview software (Version 2.8.2).

The fig. 3.7 shows the tomato Rab GTPase protein sequences used for constructing phylogenetic tree developed after the multiple sequence alignment using Clustal-X2. The neighbour joining tree (NJ) tree was bootstrapped 1000 times. The different Rab genes fall in different class, which is evident from the phylogenetic tree. Different accession numbers represent different Rab genes.

According to the Fig. 3.7 Clade 1 consist of RabD/1 having total 9 genes i.e. TC193212, TC194670, TC193170, TC193154, TC196657, TC199235, TC197849, TC203008 and TC217052. The clade 3 consists of RabC/18 having 3 genes TC210194, TC198096 and TC206013. The MSA of Rab1 and Rab18 protein sequences are shown in fig.3.6.1. Rab 1 and Rab18 are more closely related in terms of structure and usually fall in close association in the BLAST results. Clade 4 consists of RabB/2 having 4 genes TC217052, TC197445, TC198255 and TC194358. The MSA of Rab2 GTPase is shown in fig. 3.6.2.

Clade 5 consists of RabF/5 having 5 genes TC193961, TC204357, TC202099, TC209025 and TC199568. The MSA is shown in Fig3.6.3. Clade 6 consists of RabH/6 having 5 genes TC193790, TC210040, TC210789, TC213216, and TC213255. The MSA of Rab 6 is shown in the fig. 3.6.4

Clade 7 is RabG/7 having 7 genes TC197334, TC209067, TC199544, TC204750, TC206081, TC197104 and TC194527. The MSA of Rab 7 is shown in the fig.3.6.5. The clade 2 consists of Rab8/E having total of 8 genes TC193111, TC193590, TC199454, TC192325, TC191969, TC194772, TC212065, and TC207687. The MSA of Rab8 is shown in fig. 3.6.6. The sequences TC212065 is missing the initial sequences but has rest of the Rab motifs likewise in case of TC207687. This could be due to the problem with sequences and protein prediction from the library of the aligned EST sequences used to generate the TC sequences.

Finally the clade 8 consists of RabA/11 forming the largest class and group having 22 genes in total. TC202193, TC195734, TC193549, TC207615, TC200149, TC200286, TC192047, TC199565, TC195610, TC198194, TC207026, TC207267, TC205638, TC204406, TC207551, TC208438, TC208692, TC212051, TC201753, TC211111, TC202127 and TC211213. The MSA is shown in fig.3.6.7. Out of these 22 genes only 18 genes with full length sequences were used for designing the primers because of the high percentage of identity and similarity in the sequences.

It can be seen from the different MSA that each individual Rab GTPase has clear and distinct motifs. The initial motifs which are distinct to each class are shown below with the underlined amino acids of each class. The initial N-terminal protein sequences unique to each Rab GTPase class are as follows

Rab1	MNPEYDYL	FK LLLIGDSGVGK
Rab18	MEPSSYL	FK LLLIGDSGVGK
Rab2	MSYAYL	FKYIIIGDTGVGKS
Rab5	MAA+AH-NLN	AK LV L LGDMGAGKSS
Rab6	MAAVSP	LAKYK LVF LGDQSVGKT
Rab7	M - SRRR + L	LKV I I LGDSGVGK
Rab8	MAAPPARARADYDY	LIKLLLIGDSGVGKSC
Rab11	MAGYRAD+EYDY	LFK I V L I GDSGVG

The remarkable feature also lies in the C-terminus of the protein sequences. The C-terminal region is hyper variable but uniquely conserved in different classes of the Rab GTPase groups. The tails of each Rab GTPase is essential for the correct targeting of the vesicles that Rab GTPase carry to the target membrane for fusion thus enabling high specificity in the vesicle trafficking in the endo-membrane system. The characteristic C -terminal tail of the Tomato Rab GTPase is shown below

Rab 1		SGCCS S
Rab2	DGAASQG	GGCCT
Rab5	DRPAEGSRT A	SCCS
Rab6	SQSEQQG	GGCAC
Rab7	DVAASSQQR T	GGCEC
Rab8	NQPDQGAGGGQGAQKS	CCGS
Rab11	KDDVSAVKKA	GCCSS
Rab18		SGCCS

The classification and grouping of the Rab GTPases in tomato were done by comparing it with the Arabidopsis Rab GTPase genes according to Rutherford and Moore (2002) and Vernoud (2003). The MSA of Aarbidopsis and tomato Rab GTPases was done using the method mentioned earlier by ClustalX2, Bioedit, MEGA and Jalview. The Neighbour Joining (NJ) phylogenetic tree was constructed to check if the genes belonging to their respective groups also fall in with the cluster of the same group with Arabidopsis Rabs as well.

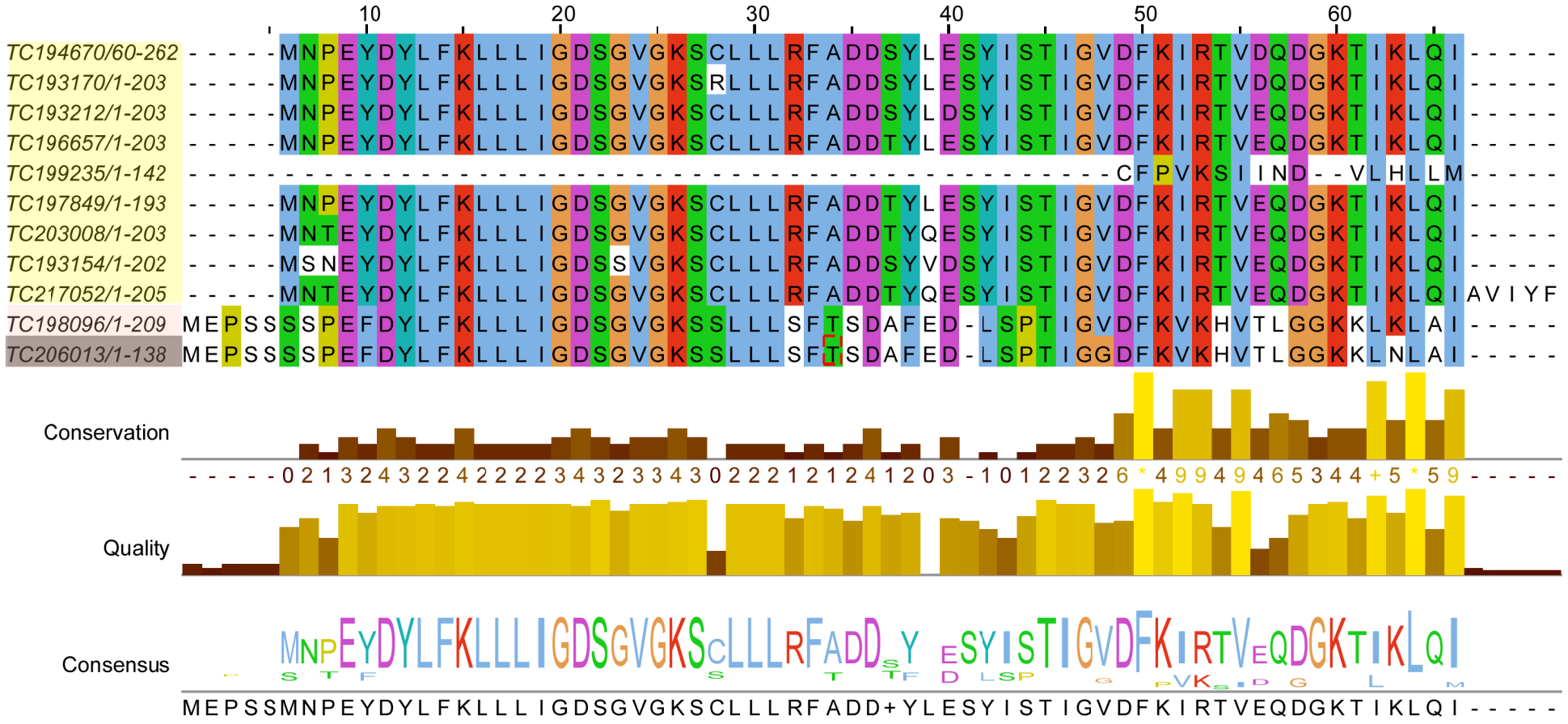


Fig. 3.6.1 The Multiple sequence Alignment of Tomato Rab1 and Rab18 GTPase with TIGR TC protein sequences Release 12.The TC198096 & TC206013 belong to Rab18 class. The Conservation plot of the protein sequences, quality of sequences and the Consensus logo has been generated be Jalview2.8.2. The blocks clearly shows the Rab F and Rab SF motifs

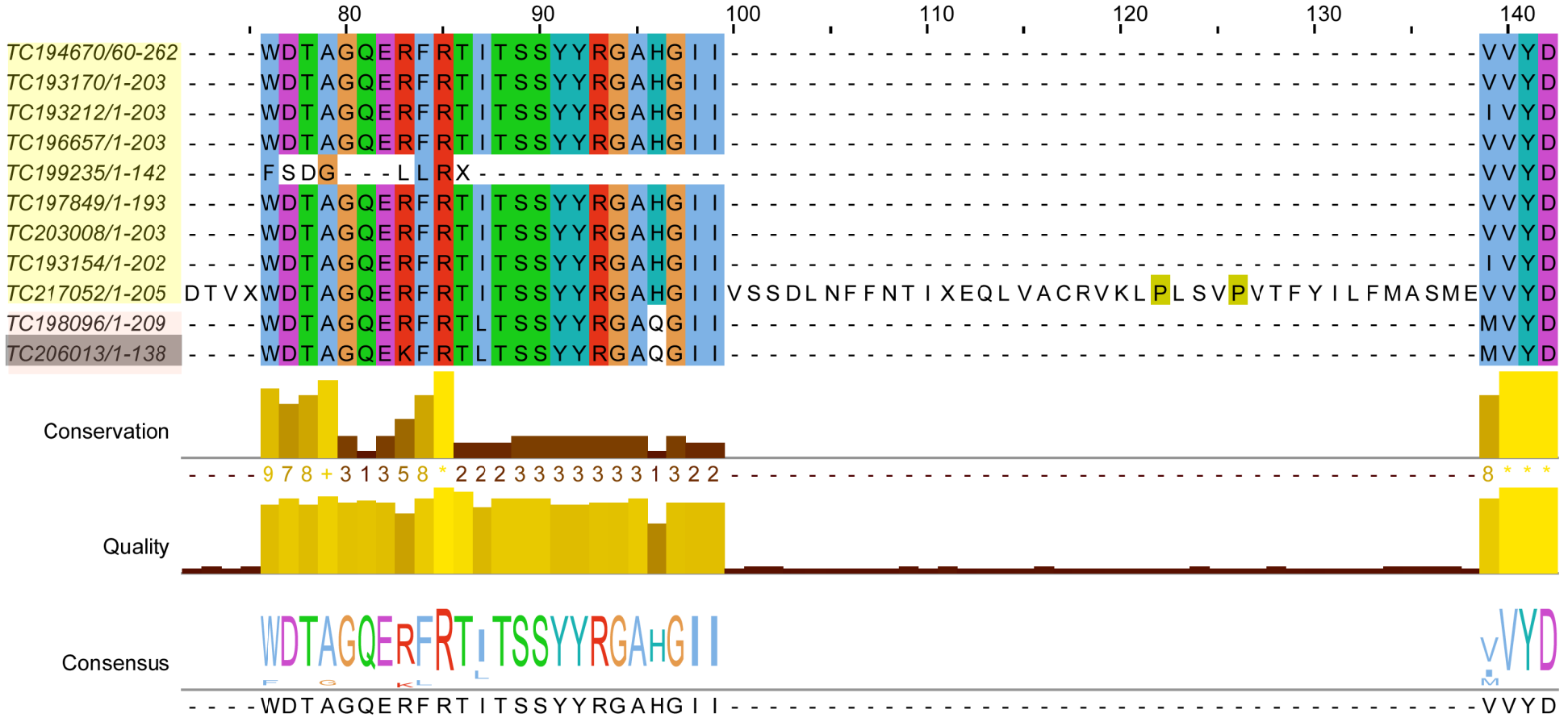


Fig. 3.6.1 The Multiple sequence Alignment of Tomato Rab1 and Rab18 GTPase Cont...

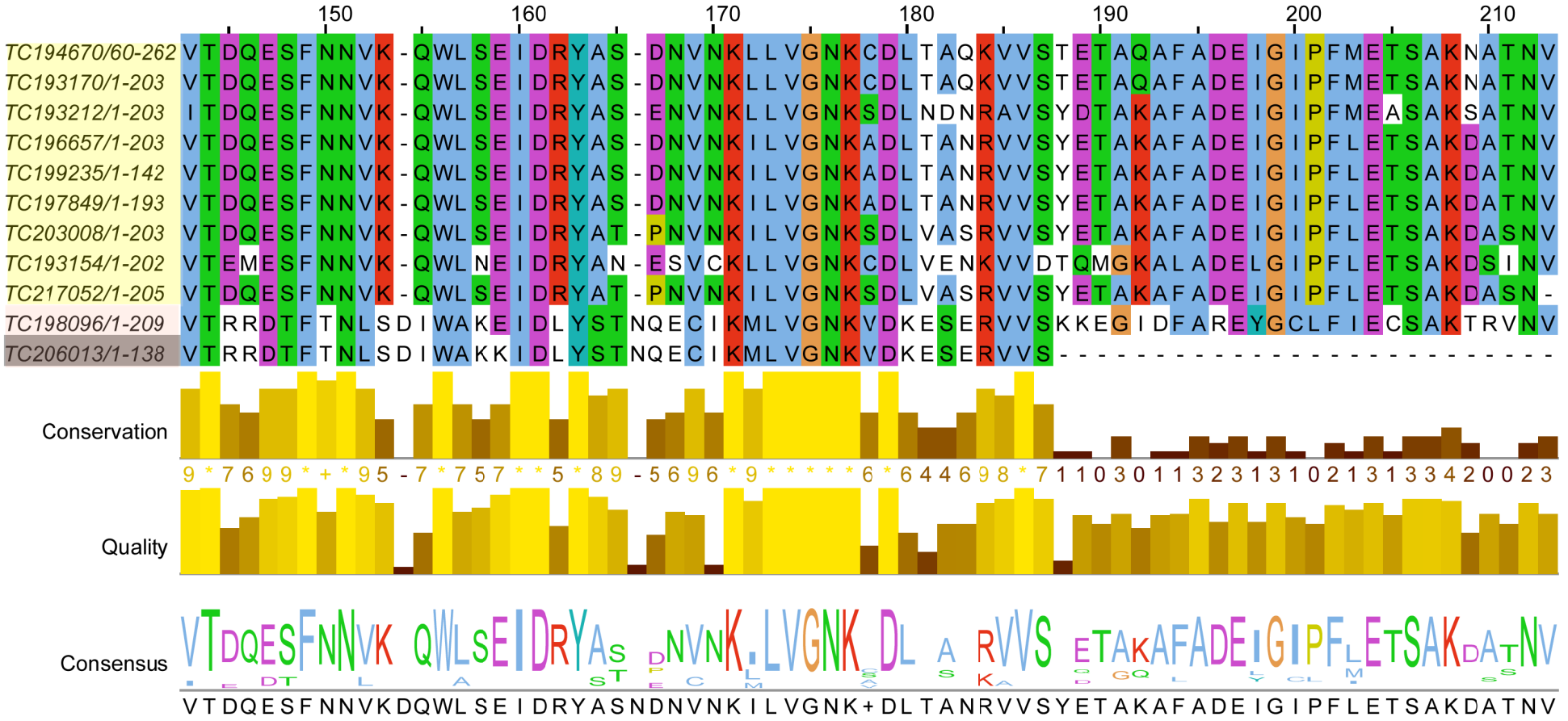


Fig. 3.6.1 The Multiple sequence Alignment of Tomato Rab1 and Rab18 GTPase cont...

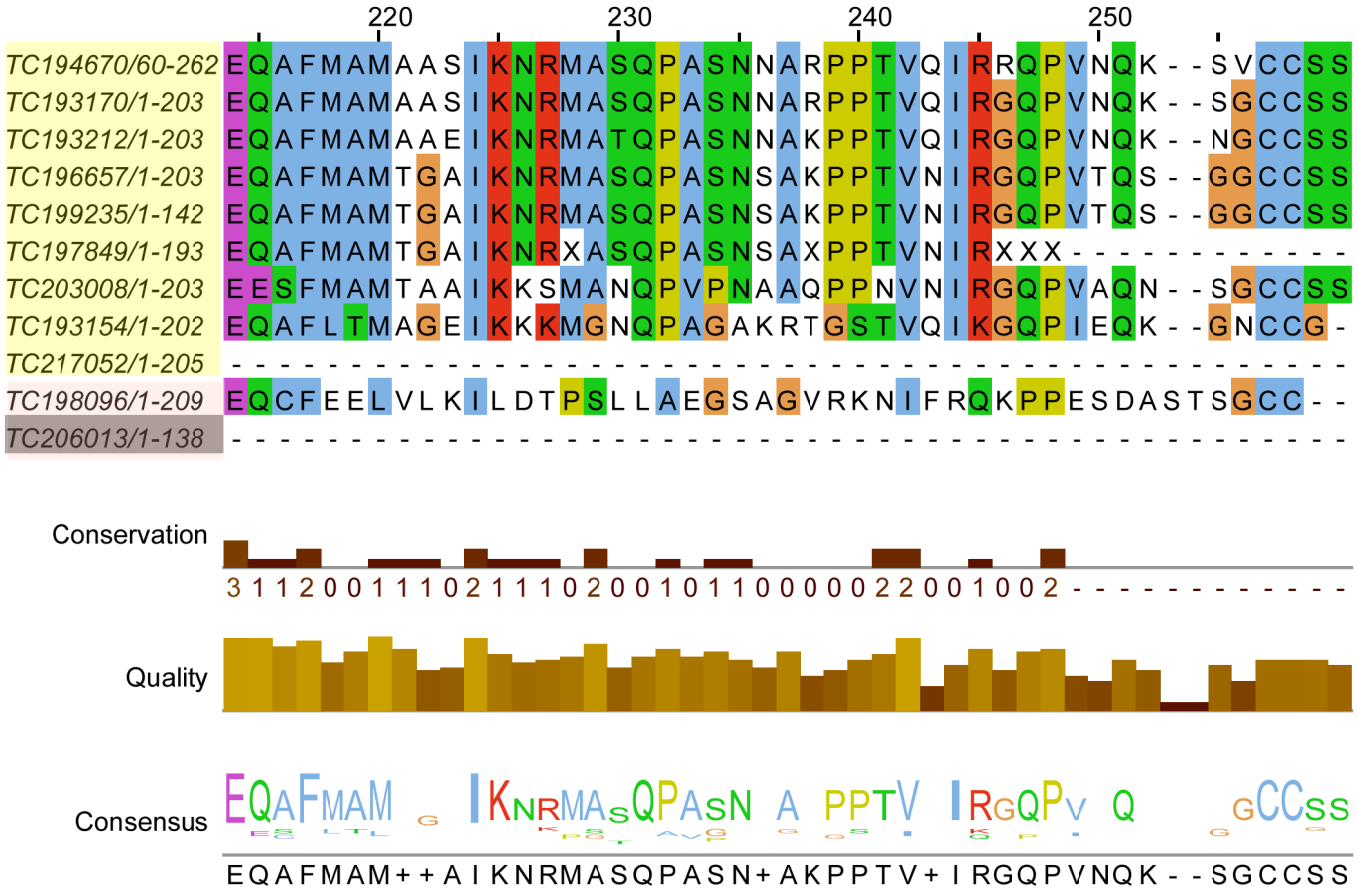


Fig. 3.6.1 The Multiple sequence Alignment of Tomato Rab1 and Rab18 GTPase cont...

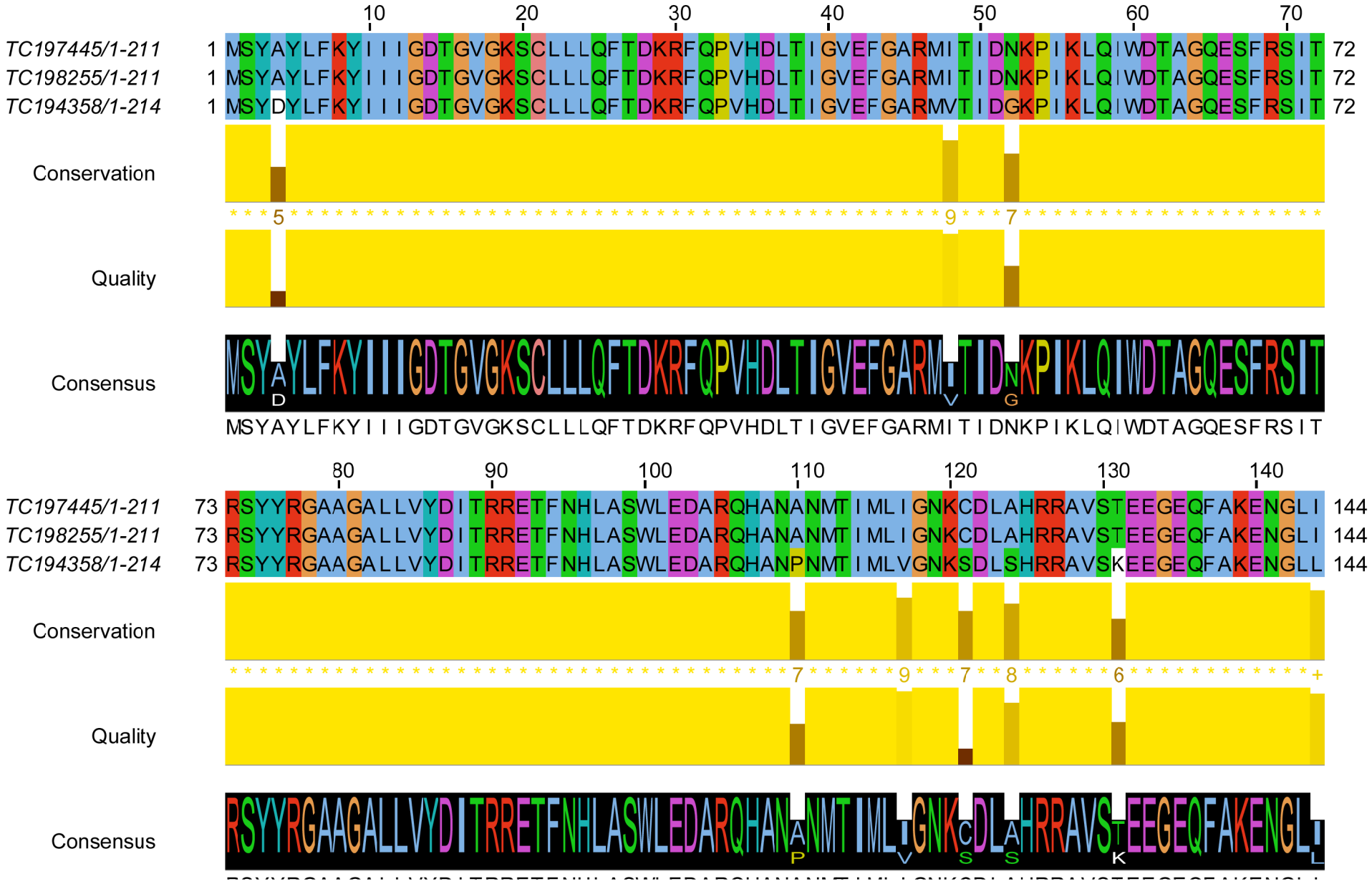


Fig. 3.6.2 The Multiple sequence Alignment of Tomato Rab2

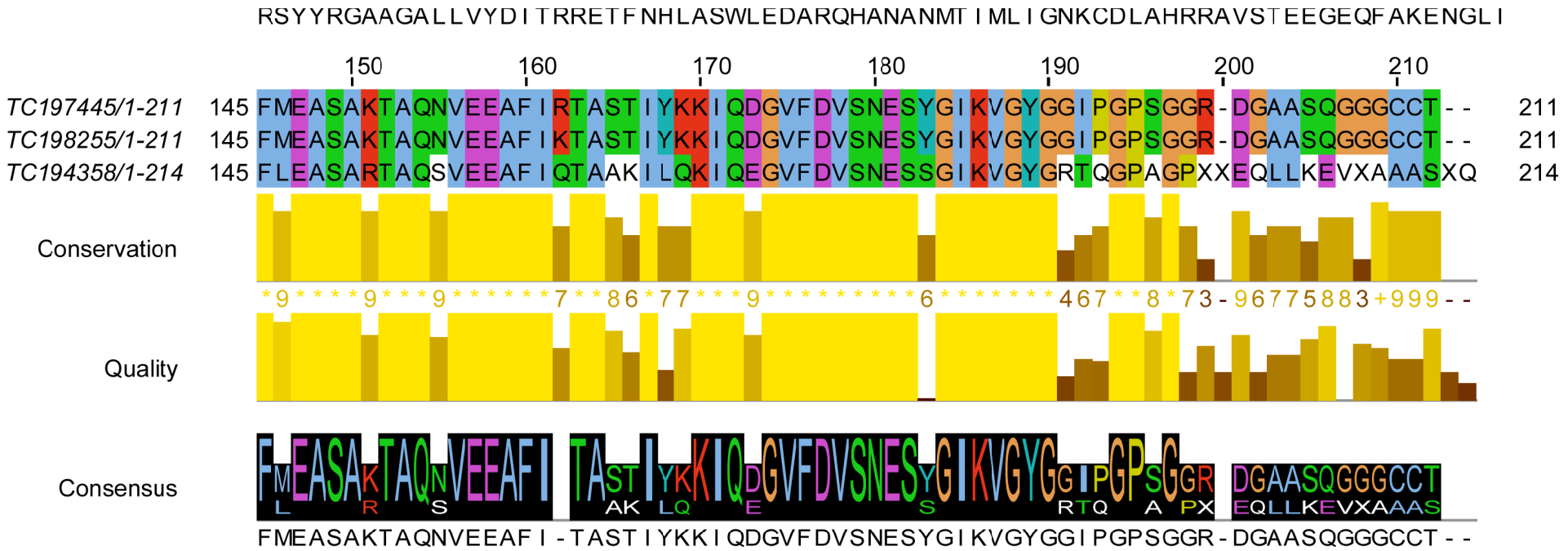


Fig. 3.6.2 The Multiple sequence Alignment of Tomato Rab2 with TIGR TC protein sequences Release 12.
The Conservation plot of the protein sequences, quality of sequences and the Consensus logo has been generated be Jalview2.8.2.
The blocks clearly shows the Rab F and Rab SF motifs

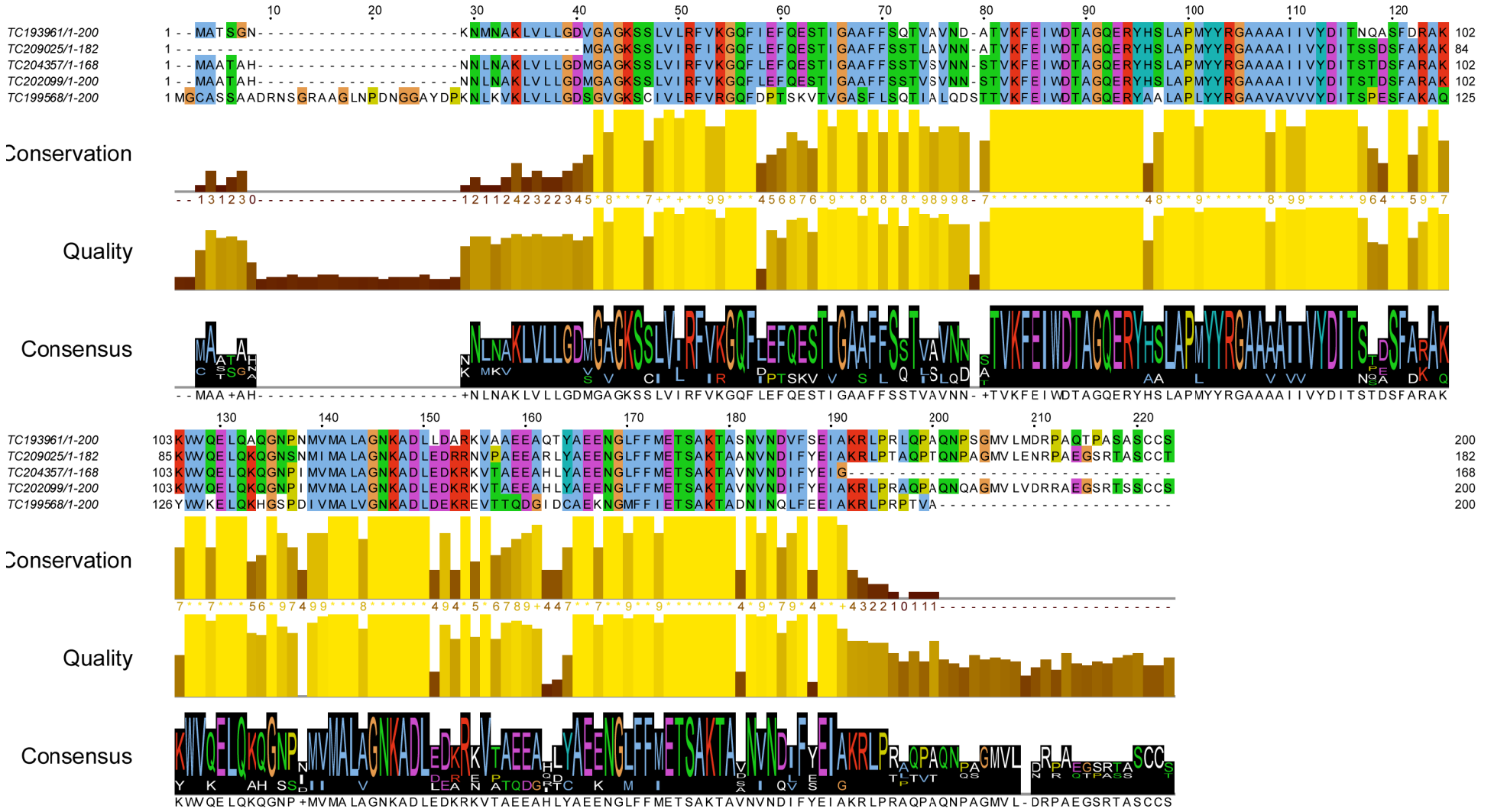


Fig. 3.6.3 The Multiple sequence Alignment of Tomato Rab5 with TIGR

TC protein sequences Release 12. The Conservation plot of the protein sequences, quality of sequences and the Consensus logo has been generated be Jalview2.8.2.

The blocks clearly shows the Rab F and Rab SF motifs

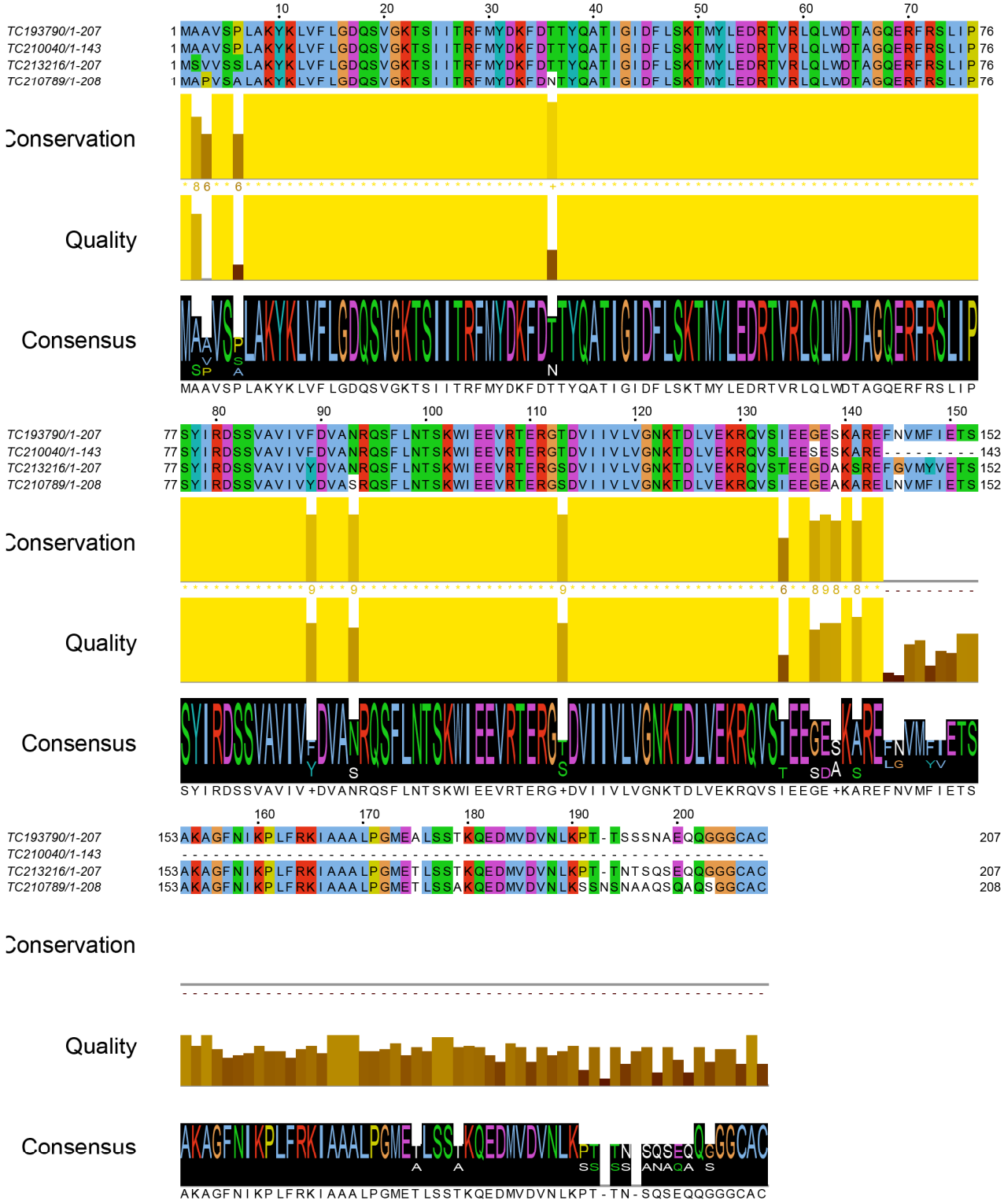


Fig. 3.6.4 The Multiple sequence Alignment of Tomato Rab6 GTPase with TIGR. TC protein sequences Release 12. The Conservation plot of the protein sequences, quality of sequences and the Consensus logo has been generated be Jalview2.8.2. The blocks clearly shows the Rab F and Rab SF motifs

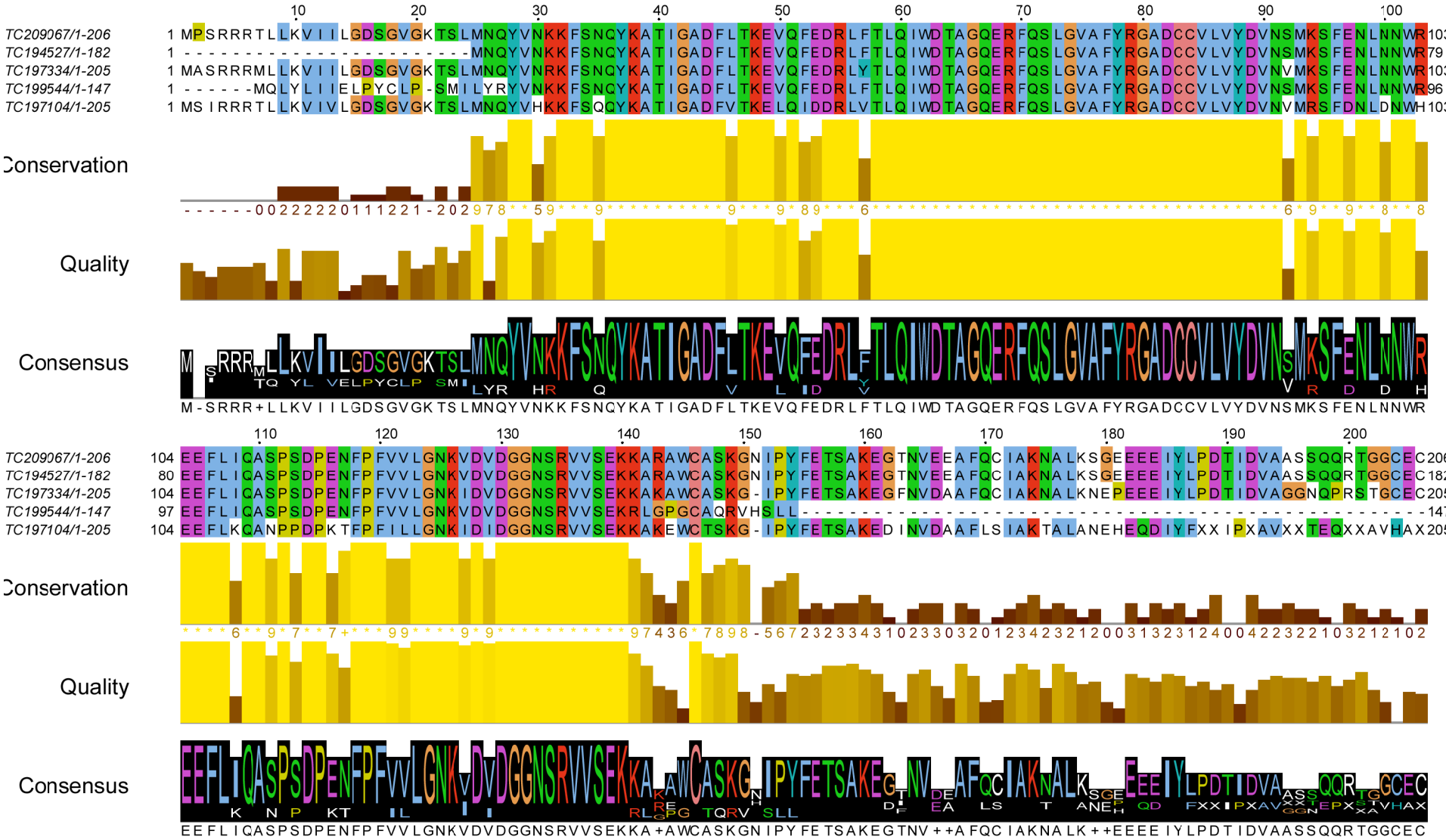


Fig. 3.6.5 The Multiple sequence Alignment of Tomato Rab7 GTPase with TIGR TC protein sequences Release 12.The Conservation plot of the protein sequences, quality of sequences and the Consensus logo has been generated be Jalview2.8.2.
The blocks clearly shows the Rab F and Rab SF motifs

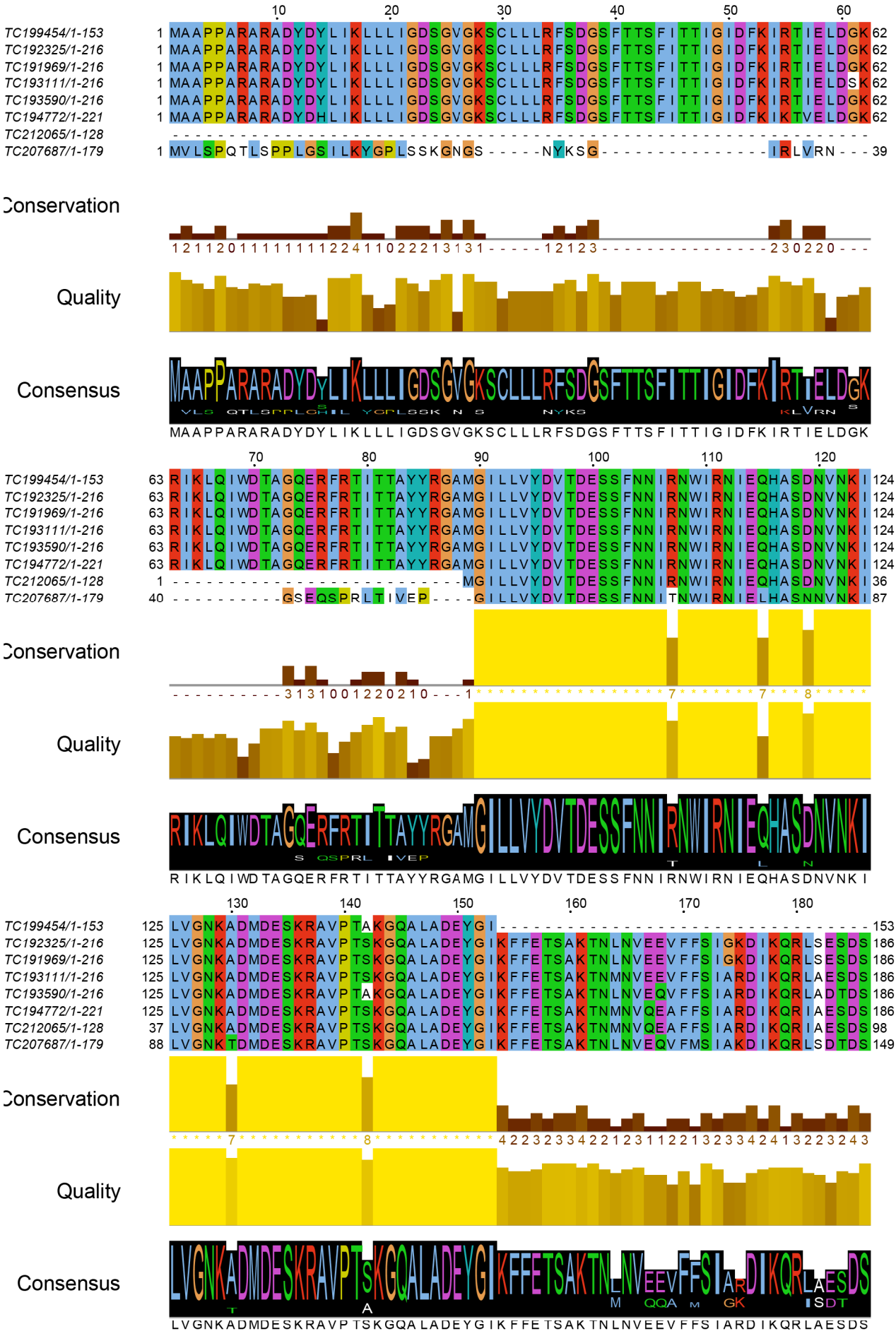


Fig. 3.6.6 The Multiple sequence Alignment of Tomato Rab8 GTPase

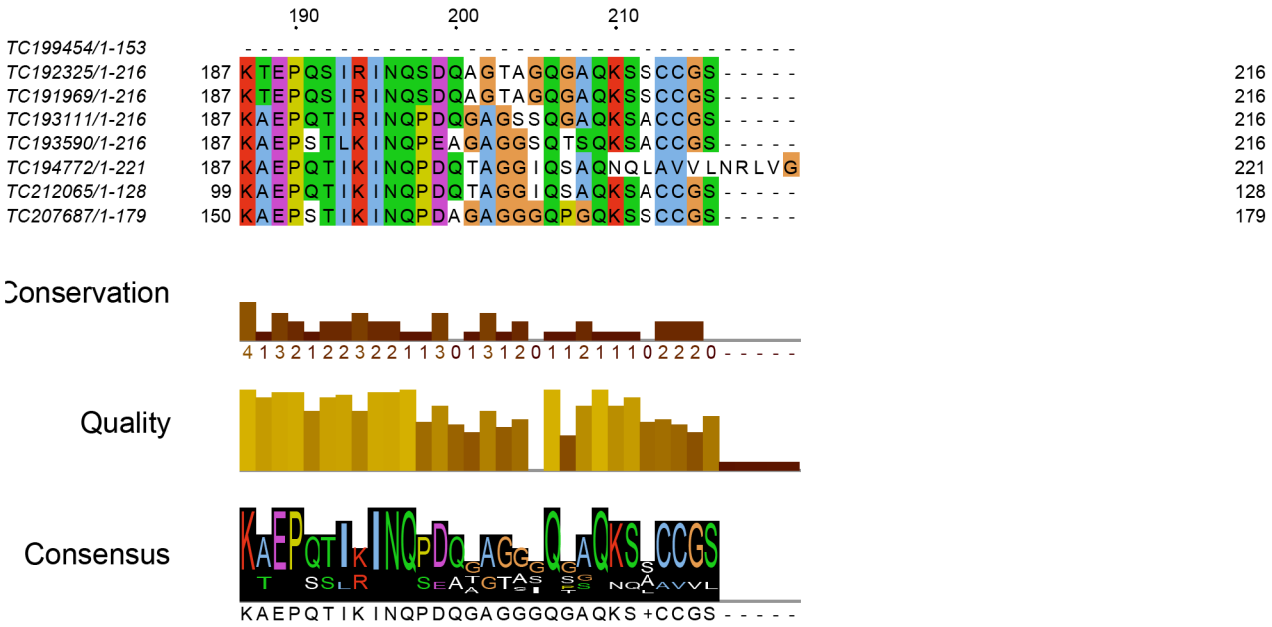


Fig. 3.6.6 The Multiple sequence Alignment of Tomato Rab8 with TIGR TC protein sequences Release 12. The Conservation plot of the protein sequences, quality of sequences and the Consensus logo has been generated be Jalview2.8.2.
The blocks clearly shows the Rab F and Rab SF motifs

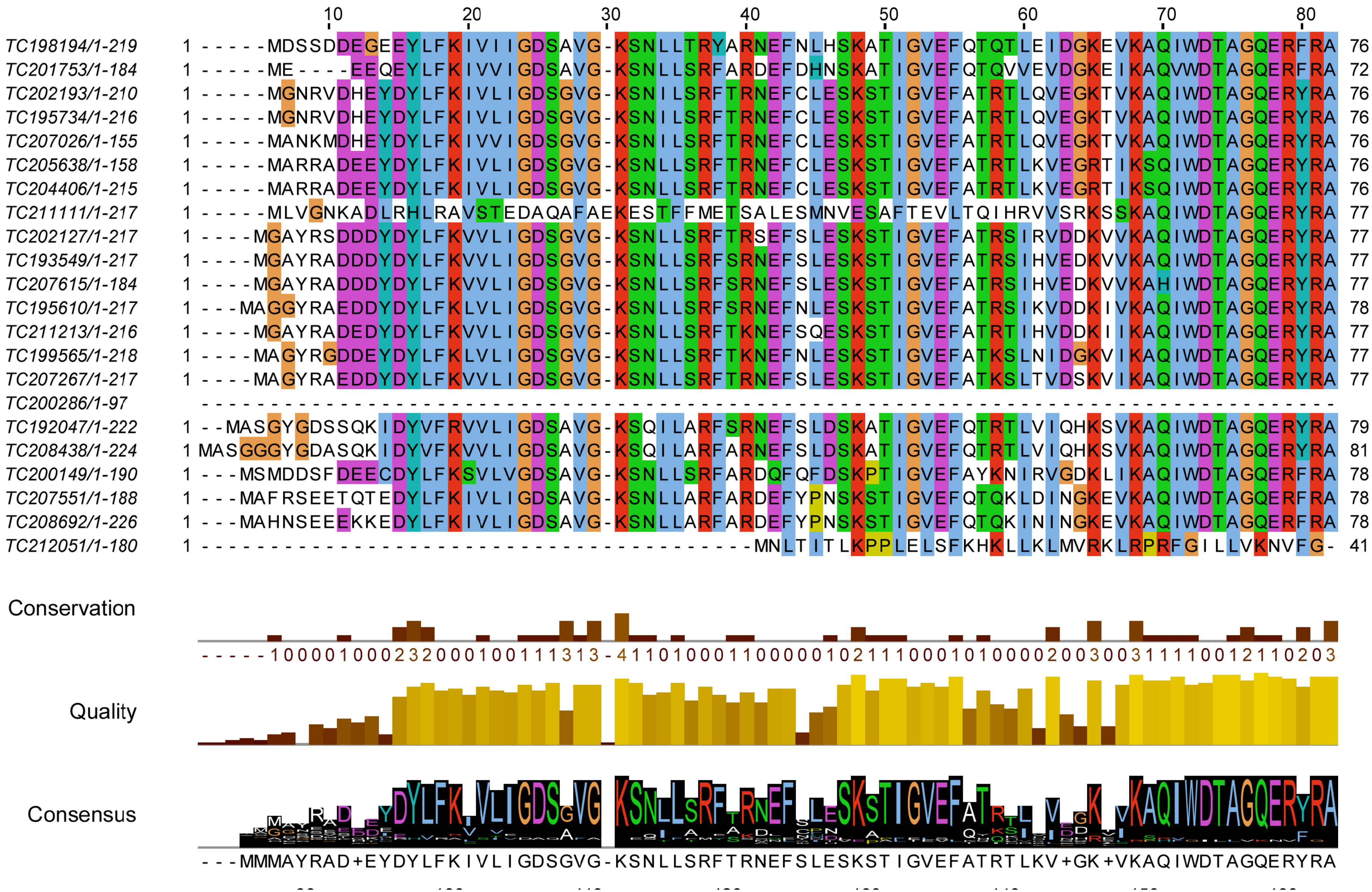


Fig. 3.6.7 The Multiple sequence Alignment of Tomato Rab11 GTPase

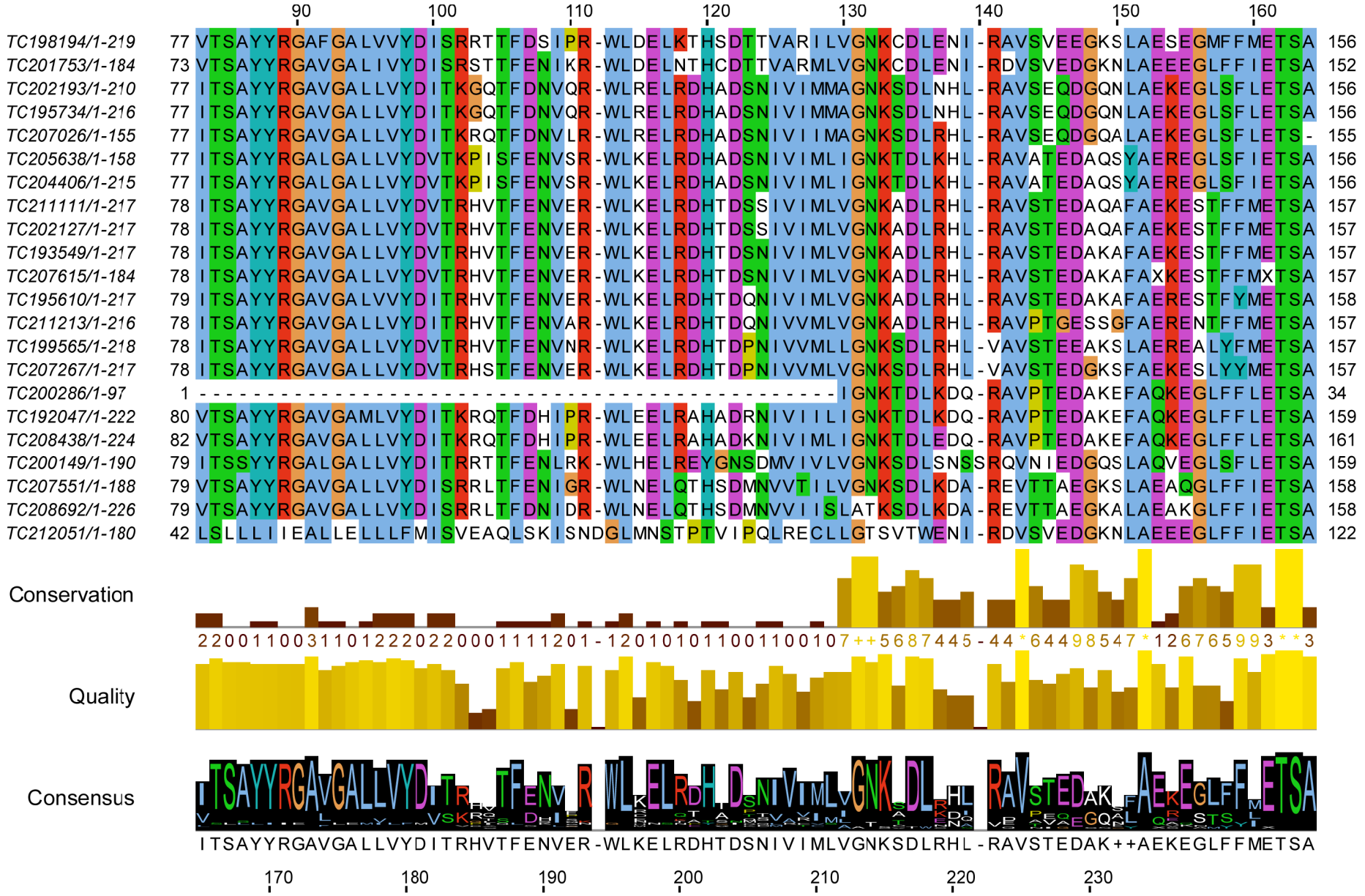


Fig. 3.6.7 The Multiple sequence Alignment of Tomato Rab11 GTPase

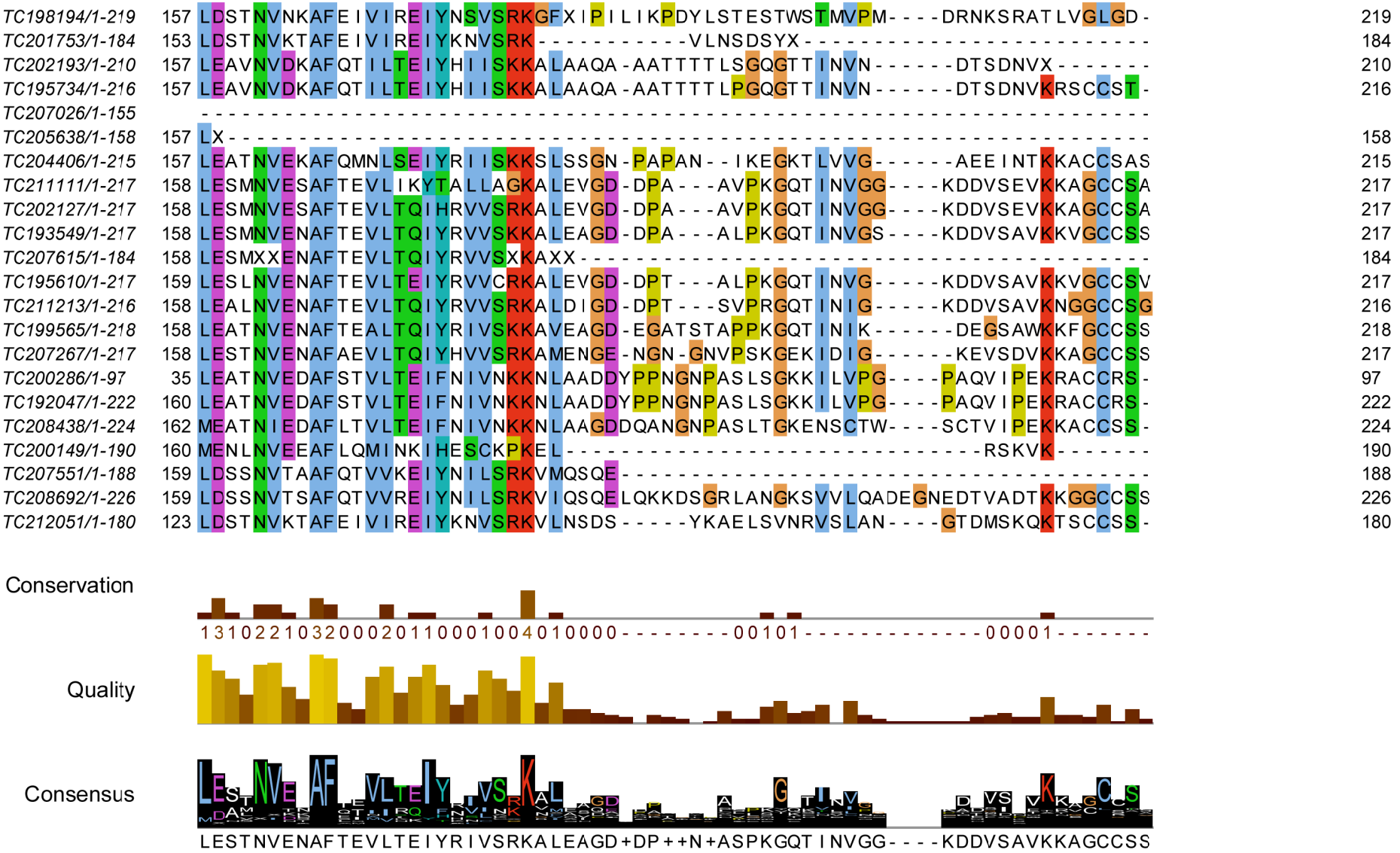


Fig. 3.6.7 The Multiple sequence Alignment of Tomato Rab11 GTPase with TIGR TC protein sequences Release 12. The Conservation plot of the protein sequences, quality of sequences and the Consensus logo has been generated be Jalview2.8.2.
The blocks clearly shows the Rab F and Rab SF motifs

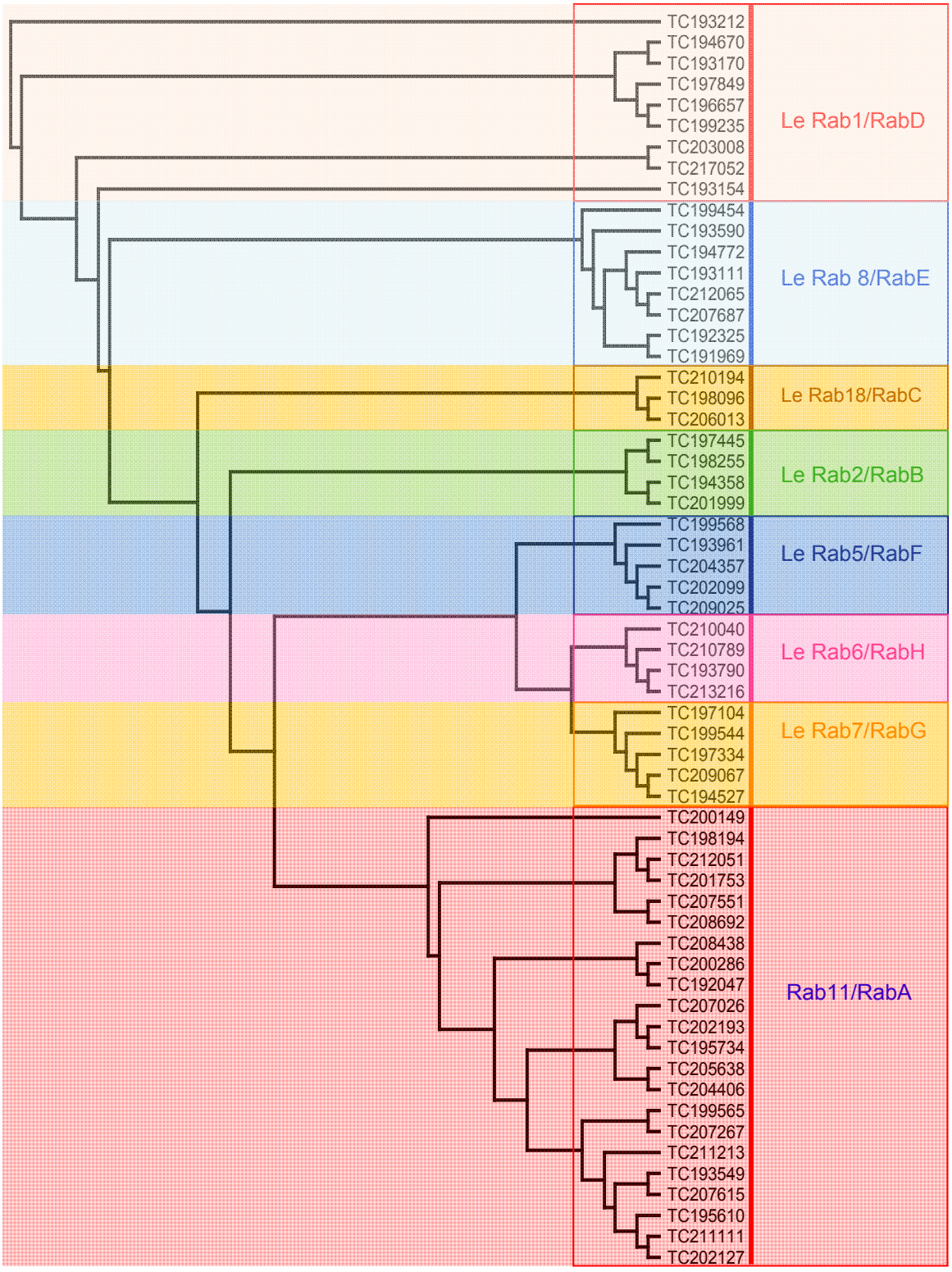


Fig 3.7 Phylogenetic tree of all Rab GTPases in tomato 12th Release TIGR TGI.

Phylogenetic tree (un-rooted) showing the topologies of the Rab GTPases in tomato based on the amino acid sequence of individual Rab genes, The neighbour joining tree is calculated with 1000 bootstrap values (not shown). The tree shows different *Rab* genes identified from TIGR TGI grouped into different classes ranging from the Le Rab 1, 8, 18, 2, 5, 6, 7 and the major class of Le Rab 11.

Phylogenetic tree of Arabidopsis Rab GTPases and tomato Rab GTPases (TIGR Release 12) is shown in fig 3.8. The tree shows different tomato and Arabidopsis Rab GTPases of different classes ranging from the Rab 1, 2, 5, 6, 7, 8, 11 and Rab18. For convenience of classification the phylogenetic tree was marked with node markers, colour coded branches and taxa of Rab GTPase sequence Ids. The results clearly indicate that the classification of tomato as shown in fig. 3.8 and Table 3.10 of tomato Rab GTPase holds true in case of comparison with the Arabidopsis Rab GTPase as well. The detailed results of individual clusters and clades (C1to C13) are as follows

Clade1-(Brown) -Rab6/RabH

Clade 1 (C1) consists of RabH/6 having 5 tomato genes TC193790, TC210040, TC210789, TC213216 and TC213255 which fall with the cluster of five Arabidopsis Rab6/H GTPases.

Clade2-(Green) -Rab5/RabF

Clade 2 (C2) consists of RabF/5 having 5 tomato genes TC193961, TC204357, TC202099, TC209025 and TC199568 which fall in cluster with three Arabidopsis Rab5 GTPases.

Clade3-(Blue) - Rab7/RabG

Clade 3 (C3) consists of RabG/7 having 7 tomato genes TC197334, TC209067, TC199544, TC204750, TC206081, TC197104 and TC194527 which fall in cluster with the 8 Arabidopsis Rab7/G GTPases.

Clade4-(Purple) - Rab8/RabE

The clade 4 (C4) consists of Rab8/E having total 8 tomato genes TC193111, TC193590, TC199454, TC192325, TC191969, TC194772, TC212065, and TC207687 falling in the same cluster with the Arabidopsis Rab8/E GTPases.

Clade5-(Sky blue) Rab1/RabD

Clade 5 (C5) consist of RabD/1 having total 9 tomato genes i.e. TC193212, TC194670, TC193170, TC193154, TC196657, TC199235, TC197849, TC203008 and TC217052 which fall in cluster with four Arabidopsis Rab1/D GTPases.

Clade6-(Fuschia) Rab18/RabC

Clade 6 (C6) consists of RabC/18 having 3 tomato genes TC210194, TC198096 and TC206013 which fall together within the same cluster of three AtRab18 GTPases.

Clade7-(Light green) Rab2/RabB

Clade 7 (C7) consists of RabB/2 having 4 tomato genes TC217052, TC197445, TC198255 and TC194358 falling in cluster with 3 Arabidopsis Rab2/B GTPases.

Table 3.10 Summary of tomato Rab GTPases (12th Release TIGR TGI) compared to Arabidopsis Rab GTPases according to phylogeny with reference to fig 3.8

Clade	Group	Gene name	AGI Code	Tomato Rab GTPase TC ids
Clade1	Rab6/RabH	*AtRABH1a*	At5g64990	TC210040
		AtRABH1b	At2g44610	TC210789
		AtRABH1c	At4g39890	TC193790
		AtRABH1d	At2g22290	TC213216
		AtRABH1e	At5g10260	TC213255
Clade2	Rab5/RabF	*AtRABF1*	At3g54840	TC199568
		AtRABF2a	At5g45130	TC193961, TC204357
		AtRABF2b	At4g19640	TC202099, TC209025
Clade3	Rab7/RabG	*AtRABG1*	At5g39620	TC199544
		AtRABG2	At2g21880	
		AtRABG3a	At4g09720	TC197104
		AtRABG3b	At1g22740	TC209067
		AtRABG3c	At3g16100	TC197334
		AtRABG3d	At1g52280	
		AtRABG3e	At1g49300	TC204750
		AtRABG3f	At3g18820	TC194527
Clade4	Rab8/RabE	*AtRABE1a*	At3g53610	TC193590, TC193111
		AtRABE1b	At5g59840	TC199454, TC192325
		AtRABE1c	At3g46060	TC191969,
		AtRABE1d	At5g03520	TC194772
		AtRABE1e	At3g09900	TC212065, TC207687
Calde5	Rab1/RabD	*AtRABD1*	At3g11730	TC193154, TC199235,
		AtRABD2a	At1g02130	TC193212,
		AtRABD2b	At5g47200	TC194670, TC193170, TC196657
		AtRABD2c	At4g17530	TC197849, TC203008, TC217052
Clade6	Rab18/RabC	*AtRABC1*	At1g43890	TC198096
		AtRABC2a	At5g03530	TC206013
		AtRABC2b	At3g09910	TC210194
Clade7	Rab2/RabB	*AtRABB1a*	At4g17160	TC201999
		AtRABB1b	At4g35860	TC194358
		AtRABB1c	At4g17170	TC198255, TC197445

A summary of Rab11/RabA GTPase group is presented in Table 3.11 according to the phylogeny (Fig3.8). Among the largest RabA/Rab11 class the following six sub groups were found and classified

Clade8 (Red) C8 consists of RabA5 sub class with three tomato genes TC207551, TC208692 and TC212051 falling in cluster with Arabidopsis AtRABA5a. Tomato gene with TC id TC201753 falls with AtRABA5b whereas TC198194 falls with AtRABA5d.

Calde9-(Sky Blue) C9 consists of RabA6 sub class having one member of tomato TC200149 in Arabidopsis RabA6 sub class.

Clade10-(Green) – C10 consists of RabA4 with 2 of the tomato genes TC208438 and TC192047 falling in cluster with AtRABA4a and one member TC200286 falls in the Arabidopsis AtRABA4d.

Calde-11-(Pink) C11 consist of Arabidopsis RabA3 with no available matching TC in tomato. The future release may shed some light if there is RabA3 in tomato as well.

Clade12-(Purple) C12 consist of RabA2 with two tomato TC205638 and TC204406 falling in cluster with AtRABA2, whereas there is one member each in RabA2b, A2c and A2d class. TC207026 in AtRABA2b, TC195734 in AtRABA2c and TC202193 falls in with AtRABA2d.

Clade13- (Blue) C13 consist of the largest of the RabA sub group. In tomato RabA1a is represented by TC199565 which falls in with Arabidopsis AtRABA1a confirming it to be RabA1a.

Tomato TC207267 falls in cluster with the Arabidopsis AtRABA1c, likewise TC211213 with AtRABA1e, TC193549 with AtRABA1f, TC207615 with AtRABA1g, and TC195610 with AtRABA1h. Arabidopsis AtRABA1i clusters together with TC211111 and TC202127. This completes the sub group classification of the tomato RabA/Rab11 GTPase according to the 12th Release of the tomato gene index of TIGR.

Table 3.11 Summary of tomato RabA/Rab11 GTPase (12th Release) compared to Arabidopsis RabA GTPases according to phylogeny with reference to fig 3.8

Clade	Group	Gene name	AGI Code	Tomato Rab GTPase TC ids
Calde13	Rab A1	*AtRABA1a*	At1g06400	TC199565
		AtRABA1b	At1g16920	
		AtRABA1c	At5g45750	TC207267
		AtRABA1d	At4g18800	
		AtRABA1e	At4g18430	TC211213
		AtRABA1f	At5g60860	TC193549
		AtRABA1g	At3g15060	TC207615
		AtRABA1h	At2g33870	TC195610
		AtRABA1i	At1g28550	TC211111, TC202127
Calde12	Rab A2	*AtRABA2a*	At1g09630	TC205638, TC204406
		AtRABA2b	At1g07410	TC207026
		AtRABA2c	At3g46830	TC195734
		AtRABA2d	At5g59150	TC202193
Calde11	Rab A3	*AtRABA3*	At1g01200	
Clade10	Rab A4	*AtRABA4a*	At5g65270	TC208438, TC192047
		AtRABA4b	At4g39990	
		AtRABA4c	At5g47960	
		AtRABA4d	At3g12160	TC200286
		AtRABA4e	At2g22390	
Clade9	Rab A6	*AtRABA6a*	At1g73640	TC200149
		AtRABA6b	At1g18200	
Clade8	Rab A5	*AtRABA5a*	At5g47520	TC207551, TC208692, TC212051
		AtRABA5b	At3g07410	TC201753
		AtRABA5c	At2g43130	
		AtRABA5d	At2g31680	TC198194
		AtRABA5e	At1g05810	

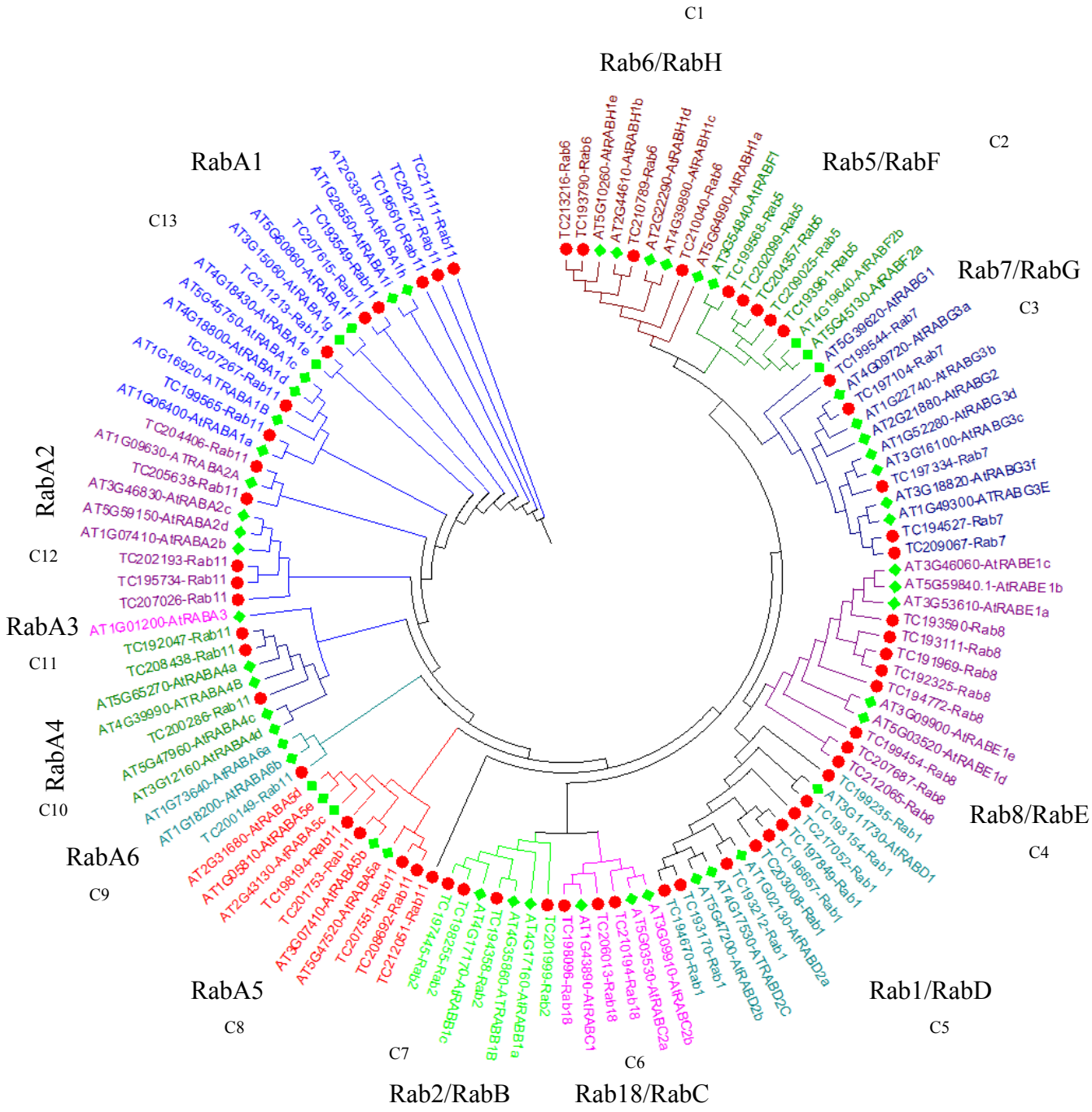


Fig 3.8 Phylogenetic tree of LeRab GTPase v/s AtRab GTPases (TIGR Release 12)
Phylogenetic NJ Tree of Le v/s At Rab GTPase with Bootstrap test (1000 bootstrap value not shown) according to Release 12 of DFCI tomato gene index based on the amino acid sequence of individual Rabs. The tree shows different tomato and Arabidopsis Rab GTPases of different classes ranging from Rab1, 2, 5, 6, 7, 8 and Rab11. Rab11/Rab A group is further divided into RabA1-A6.
Node Markers: ● The red filled circle represents tomato sequence Ids;
◆ The light green filled diamond represents the Arabidopsis sequence ids.
Colour codes for branches and taxa: Clade1: Brown (Rab6/RabH), Clade2-green (Rab5/RabF); Clade3-Blue (Rab7/RabG); Clade4-Purple (Rab8/RabE); Clade5-Sky blue (Rab1/RabD); Clade6-Fuschia (Rab18/RabC); Clade7-light green (Rab2/RabB); Clade8-Blue branch (Rab11/RabA). Among the RabA/Rab11 sub-groups the following branch and taxa colour code was used: Red-RabA5, Sky Blue-RabA6, Green-RabA4, Pink- RabA3, Purple-RabA2 and the sub group of RabA1 is represented by blue colour branches and taxon labels.
Clades: C1 to C13 as described in the text; C8 to C13 refers to RabA group

Table 3.12 New Tomato Rab GTPase TIGR DFCI TGI release 13 (April 2010)

Class of Rab GTPase	Release12 Jul 2008	New TC Release 13 April 2010
RabD/Rab1	TC193212	TC218008
	TC194670	TC219445
	TC193170	TC217961
	TC193154	TC217946
	TC196657	TC230012 TC218685 TC217784
	TC199235	TC224211
	TC197849	TC222748
	TC203008	TC228595
	TC217052	TC245198
RabC/Rab18	TC198096	TC223016
	TC206013	TC232260
	TC210194	TC235984
RabB/Rab2	TC197445	TC239055
	TC198255	TC223185
	TC194358	TC219185
	TC201999	TC242604
RabF/Rab5	TC193961	TC218706
	TC204357	TC227481
	TC202099	TC234866
	TC209025	TC235767 TC224486
	TC199568	TC224570
RabH/Rab6	TC193790	TC218546
	TC210040	TC229699
	TC210789	TC237903
	TC213216	TC240704
	TC213255	TC240746 Incomplete ORF (95 aa)
RabG/Rab 7	TC197334	TC222197
	TC209067	TC217468
	TC199544	TC224535
	TC204750	TC230711
	TC206081	TC220556
	TC197104	TC220556
	TC194527	TC217468 Repeat
RabE/Rab 8	TC193111	TC217915
	TC193590	TC218364
	TC199454	TC224448
	TC192325	TC217391
	TC191969	TC224023
	TC194772	TC219550
	TC212065	TC239367 Distinctive Rab8 N-termi Motif with MGIL
	TC207687	TC234213

Class of Rab GTPase	Release12 Jul 2008	New TC Release 13 April 2010	
RabA/Rab11	TC202193	TC227595	RabA2
	TC195734	TC220527	RabA2
	TC193549	TC223336	RabA1
	TC207615	TC234136	RabA1
	TC200149	TC223058	RabA6
	TC200286	TC225354	RabA6
	TC192047	TC225814	RabA3
	TC199565	TC224574	RabA1
	TC195610	TC220390	RabA1
	TC198194	TC223129	RabA5
	TC207026	TC233465	RabA5
	TC207267	TC233734	RabA1
	TC205638	TC231813	RabA1
	TC204406	TC230320	RabA5
	TC207551	TC234079	RabA4
	TC208438	TC235051	RabA5
	TC208692	TC235365	RabA5
	TC212051	TC239355	RabA5
	TC201753	TC227076	RabA5
	TC211111	TC224630	RabA5
	TC202127	TC227506	RabA1
	TC211213	TC238379	RabA1

The additional TC in the 13th Release of TGI indicates that the TC of previous 12th Release were split into new TC. This is also highlighted in the sequence annotations and the BLAST outputs (see supplemental files for BLAST results). Caution has to be exercised as several newly assigned TC sometimes have incomplete ORFs and the sequences have errors due to automated annotation scripts. It is important to note that there is no need to BLAST OLD TC to the NEW TC; since the new release only indicates the change in names of the older TC as is the case with naming of TC in the newer version. The ORFs of new TC were individually inspected for errors and incomplete ORFs were removed.

The table no. 3.12 shows the list of old (12th Release) and new (13th Release) TC ids. According to the 13th Release the sequence analysis revealed that there are 22 RabA/Rab11 GTPase in total with eight RabA1 in tomato. The Blast results with the best hits of the new Rab GTPases according to the 13th release of the TIGR DFCI tomato gene index are shown in table 3.13. There are few more genes added and the TC ids have been changed. Only those sequences were taken for the analysis for MSA which had complete AA sequence length and all the Rab family and subfamily specific motifs. Please see the Appendix and supplemental files for the complete MSA of both Arabidopsis and tomato Rab GTPases.

Table 3.13 BLAST results of Arabidopsis Rab11/RabA GTPase with the TIGR TGI release 13.

Arabidopsis Rab Name	AGI Code	Old TC Release 12	New TC Release 13	E -Value	Score	Identities	Positives	Frames	ORF Amino Acid Length
ATRABA1A	AT1G06400	TC199565	TC224574	5.0e-87	867 310.3 bits	169/219 77%	187/219 85%	+3	135 .. 790 5' end: 135 3' end: 790 218 aa
ATRABA1B	AT1G16920	TC207267	TC233734	2.3e-92	918 (328.2 bits)	177/218 (81%)	202/218 (92%)	+3	171 .. 823 5' end: 171 3' end: 823 217 aa
		TC205638	TC231813					+2	206 .. 677 5' end: 206 3' end: 677 156 aa
AtRABA1c	AT5G45750	TC207267	TC233734	1.1e-94	940 (336.0 bits),	186/218 (85%),	198/218 (90%),	+3	
ATRABA1D	AT4G18800	TC207267	TC233734	3.0e-99	983 (351.1 bits),	195/218 (89%),	207/218 (94%),	+3	171 .. 823 5' end: 171 3' end: 823 217 aa
AtRABA1e	AT4G18430	TC193549	TC223336	3.6e-96	954 340.9 bits	177/218 81%	206/218 94%	+2	
AtRABA1e	AT4G18430	TC211213	TC238379	1.4e-95	949 (339.1 bits)	183/218 (83%)	202/218 (92%)	+1	
AtRABA1f	AT5G60860	TC193549	TC223336	4.0e-106	1048 (374.0 bits),	204/218 (93%),	215/218 (98%),	+2	
AtRABA1f	AT5G60860	TC207615	TC234136					+2	209 .. 758 5' end: 209 3' end: 758 182 aa
AtRABA1g	AT3G15060	TC193549	TC223336	3.3e-102	1011 (360.9 bits),	196/218 (89%),	211/218 (96%),	+2	
AtRABA1g	AT3G15060	TC195610	TC220390	1.4e-96	959 342.6 bits	185/213 86%	204/213 95%	+3	93 .. 745 5' end: 93 3' end: 745 217 aa
AtRABA1g	AT3G15060	TC202127	TC227506	1.5e-98	977 (349.0 bits)	187/218 (85%)	210/218 (96%)	+1	
		TC202127	TC227506					+1	118 .. 770 5' end: 118 3' end: 770 217 aa
AtRABA1g	AT3G15060	TC211213	TC238379	4.2e-96	954 (340.9 bits)	184/218 (84%)	203/218 (93%)	+1	
		TC211213	TC238379					+1	145 .. 794 5' end: 145 3' end: 794 216 aa
ArRABA1h	AT2G33870	TC193549	TC223336	5.8e-98	971 (346.9 bits),	188/219 (85%),	207/219 (94%),	+2	
AtRABA1i	AT1G28550	TC193549	TC223336	9.1e-100	988 352.9 bits	191/219 87%	210/219 95%	+2	86 .. 738 5' end: 86 3' end: 738 217 aa
ATRABA2C	AT3G46830	TC202193	TC227595	3.0e-89	890 318.4 bits	177/207 85%	186/207 89%	+1	115 .. 742 5' end: 115 3' end: 742 208 aa
ATRABA2C	AT3G46830	TC195734	TC220527	1.1e-93	932 333.1 bits	184/218 84%	195/218 89%	+3	

ATRABA2D	AT5G59150	TC195734	TC220527	3.2e-92	918 328.2 bits	181/218 83%	192/218 88%	+3	
ATRABA3	AT1G01200	TC192047	TC225814	4.6e-62	633 227.9 bits	131/217 60%	166/217 76%	+2	74 .. 741 5' end: 74 3' end: 741 222 aa
AtRABA4a	AT5G65270	TC208438	TC235051	1.3e-100	996 (355.7 bits)	196/227 (86%),	209/227 (92%),	+3	
AtRABA4a	AT5G65270	TC208438	TC235051	1.3e-100	996 (355.7 bits)	196/227 (86%)	209/227 (92%)	**+3**	177 .. 850 5' end: 177 3' end: 850 224 aa
ATRABA4B	AT4G39990	TC208438	TC235051	5.3e-88	877 (313.8 bits)	169/213 (79%)	195/213 (91%),	+3	
ATRABA4C	AT5G47960	TC208438	TC235051	2.3e-80	805 (288.4 bits)	158/215 (73%)	185/215 (86%),	+3	
ATRABA4D	AT3G12160	TC208438	TC235051	4.0e-83	831 (297.6 bits)	158/203 (77%)	185/203 (91%)	+3	
AtRABA5a	AT5G47520	TC207551	TC234079	3.6e-86	Score = 859 (307.4 bits)	170/188 (90%)	180/188 (95%)	+3	
AtRABA5a	AT5G47520	TC208692	TC235365	3.0e-90	581 (209.6 bits)	114/128 (89%),	120/128 (93%),	+2	1339 .. 661 5' end: 1339 3' end: 661 225 aa
		TC212051	TC239355					+1	94 .. 635 5' end: 94 3' end: 635 180 aa
AtRABA5b	AT3G07410	TC198194	TC223129	1.8e-79	797 285.6 bits	160/219 (73%),	186/219 (84%),	+1	
ATRABA5C	AT2G43130	TC198194	TC223129	4.2e-87	869 (311.0 bits)	172/218 (78%)	196/218 (89%)	+1	
ATRABA5C	AT2G43130	TC201753	TC227076	5.4e-78	784 (281.0 bits),	149/182 (81%)	171/182 (93%)	+1	154 .. 704 5' end: 154 3' end: 704 183 aa
		TC211111	TC224630					ESTScan (+)	1 .. 517 5' end: 1 3' end: 517 170 aa
		TC211111	TC224630	incomplete ORF				+3	228 .. 517 5' end: 228 3' end: 517 96 aa
AtRABA5d	AT2G31680	TC198194	TC223129	1.8e-86	863 (308.9 bits)	171/201 (85%),	189/201 (94%)	+1	
ATRABA5E	AT1G05810	TC198194	TC223129	4.6e-62	633 (227.9 bits)	131/217 (60%)	166/217 (76%)	+1	76 .. 728 5' end: 76 3' end: 728 217 aa
		TC207026	TC233465					+3	57 .. 521 5' end: 57 3' end: 521 154 aa
AtRABA6a	AT1G73640	TC200149	TC223058	4.1e-75	755 270.8 bits	151/233 64%	189/233 81%	+1	
AtRABA6b	AT1G18200	TC200149	TC223058	4.2e-73	736 264.1 bits	152/231 65%	186/231 80%	+1	211 .. 887 5' end: 211 3' end: 887 225 aa
		TC200286	TC225354						

Table shows the Arabidopsis Rab GTPase used as query for BLAST into the TIGR database following the 13th TGI Release. The blank rows in the table indicate that although no E values were assigned but ORFs were visually inspected and used for alignment to make sure it falls in the RAB GTPases group. For more details please check the additional supplemental files (Individual notepad- *.txt files) for the BLAST hit results and ORF sequence information.

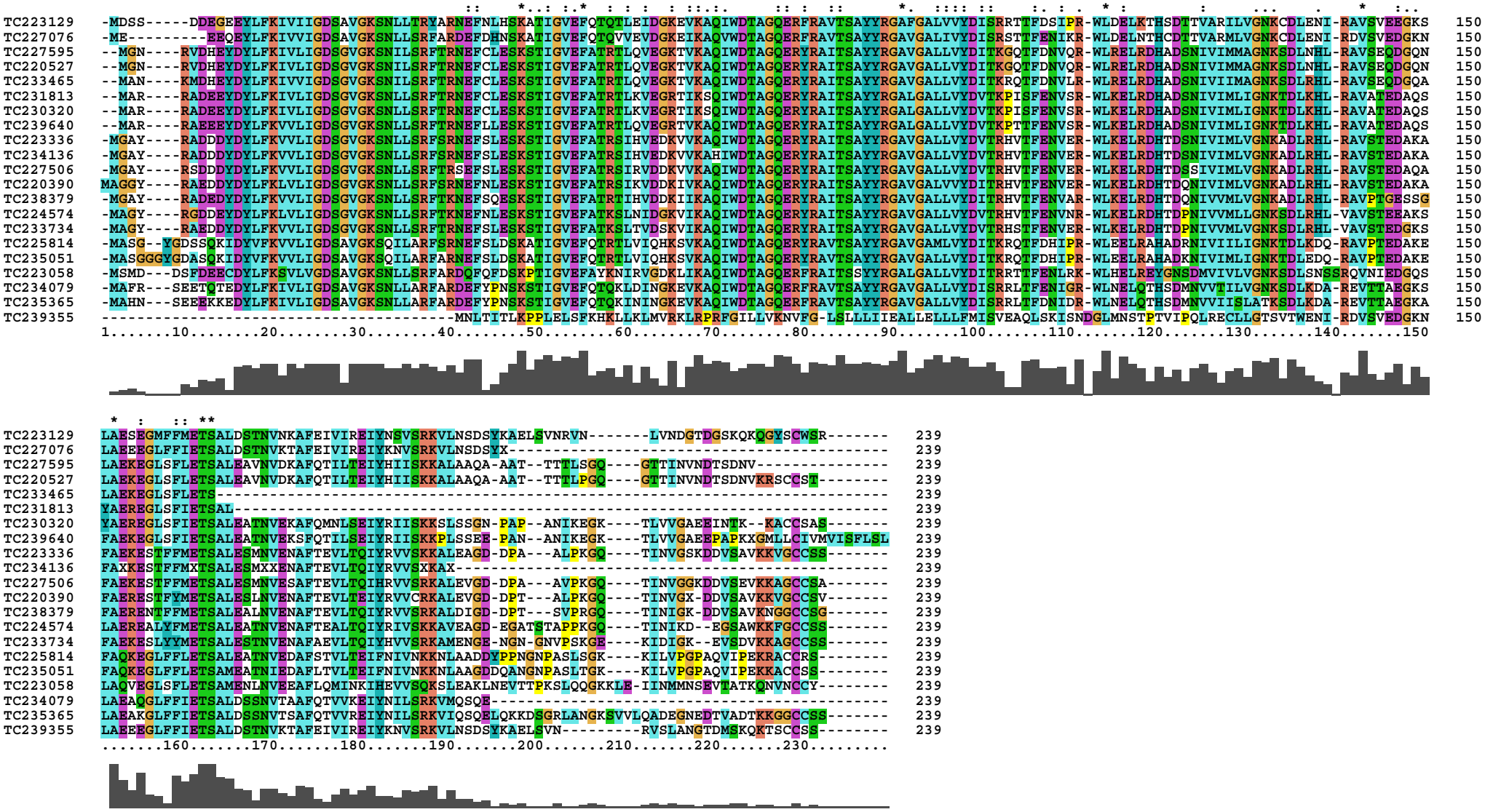


Fig. 3.9.1 The Multiple sequence Alignment of Tomato Rab11 GTPase with TIGR TC Release 13.
Protein sequences are aligned by ClustalX2

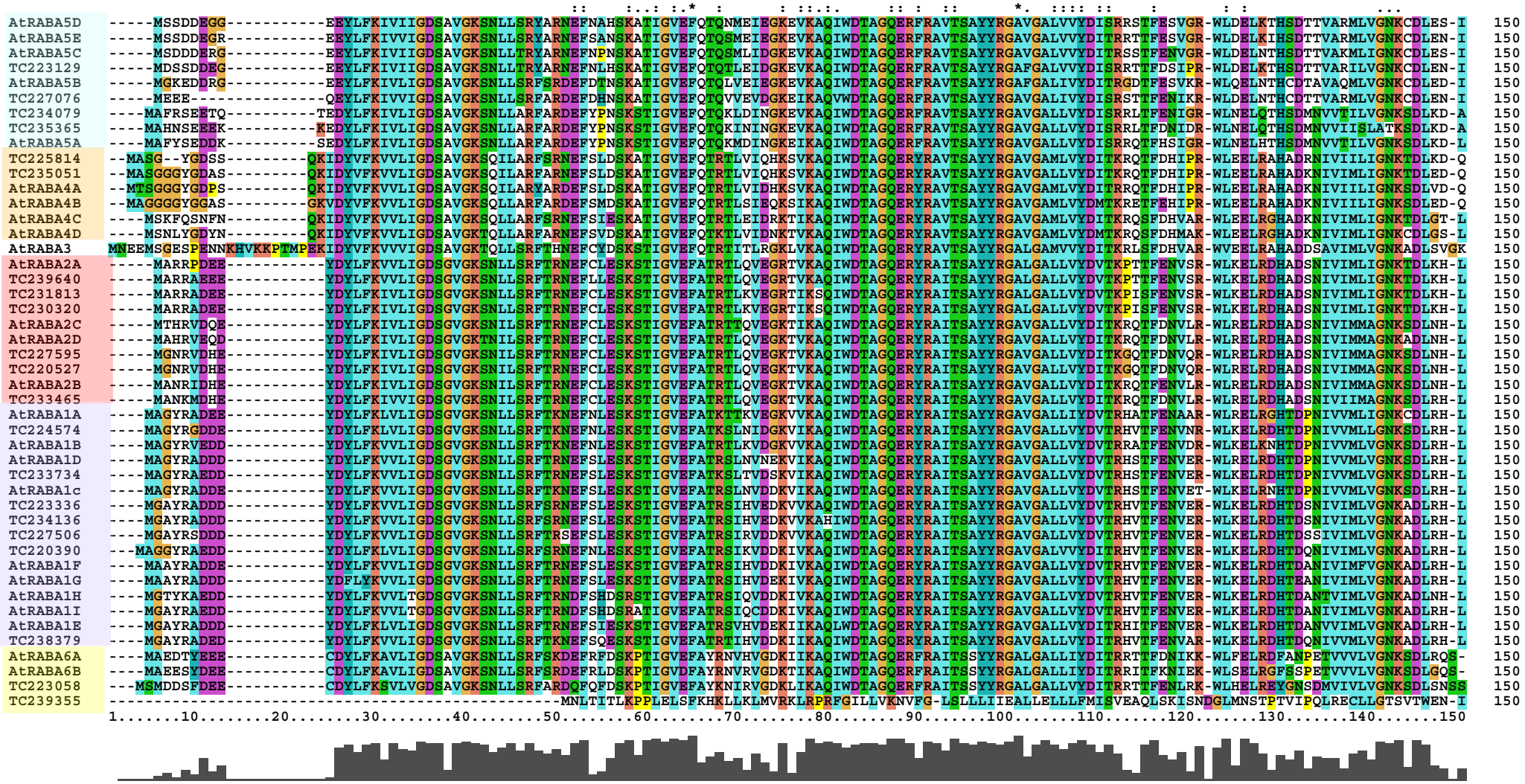


Fig. 3.9.2 The Multiple sequence Alignment of Arabidopsis and Tomato Rab11 GTPase with TIGR TC Release 13. Protein sequences are aligned by ClustalX2. and printed from postscript files. The black bars below the alignment shows the group conservation consensus histogram

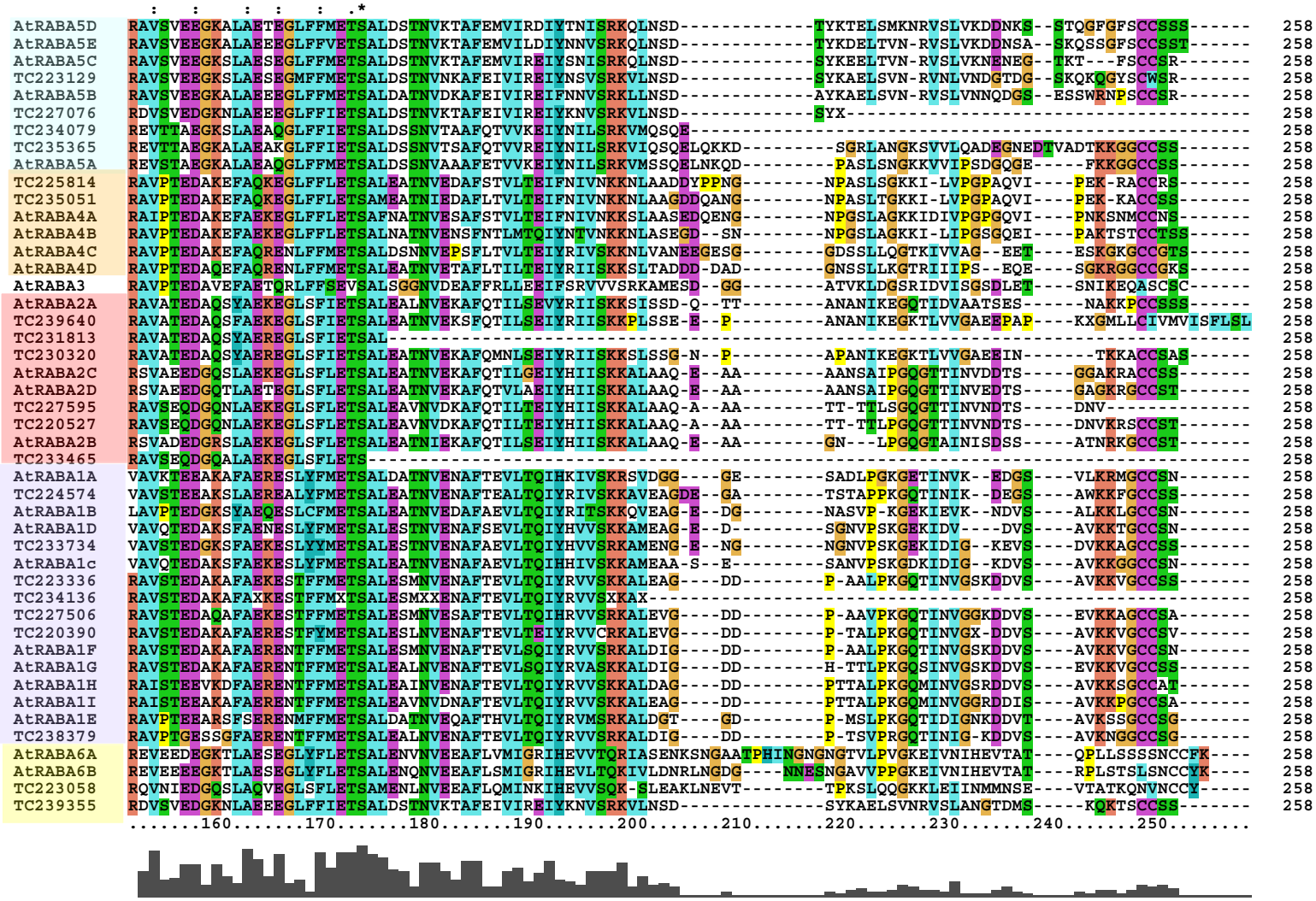


Fig. 3.9.2 The Multiple sequence Alignment of Arabidopsis and Tomato Rab11 GTPase with TIGR TC Release 13 cont...

Figure 3.10 shows the phylogenetic analysis of the individual tomato Rab GTPase (13th Release TIGR TGI) the individual Rab members fall together in the same cluster as inferred from the tree. In tomato there are nine genes in Rab1/RabD; nine in Rab8/RabE; three in Rab18/RabC; four in Rab2/RabB, six in Rab5/RabF; five in Rab6/RabH; four in Rab7/RabG and twenty one members of Rab11/RabA all falling in the individual clusters and clade (fig.3.11). In order to validate the classification of tomato Rab GTPase a phylogenetic tree with Arabidopsis Rab GTPase as reference was calculated. Figure 3.12 shows the Phylogenetic tree of Arabidopsis and Tomato Rab 1,2,5,6,7,8 and Rab18 GTPase (13th Release of the TIGR DFCI TGI). These results validate the phylogeny of the individual tomato Rab GTPase (fig.3.12). The Clade1 (Brown) show four of the tomato Rab7/RabG GTPase fall within the same cluster of eight Arabidopsis Rab7 group. Clade2-(Green) consists of Rab6/RabH GTPase and five of tomato Rab 6/RabH falls together with five members of AtRab6/RabH. Clade3-(Fuschia) consist of six tomato Rab5/RabF which fall with 3 of At Rab5/RabF. Clade4-(Navy Blue) consists of three tomato Rab18/RabC falling in the same cluster with three of AtRab18/RabC. Clade5-(Purple) consist of four tomato and four Arabidopsis Rab2/RabB members. Clade6-(Red) had the most surprising results as a total of nine members of tomato Rab1/RabD group fall with four of Arabidopsis Rab1/RabD. The unusually high number of the representative tomatoRab1/RabD GTPase may indicate the duplication of Rab1 or putative alleles or even the same sequence with different TC ids. The last Clade7-(Blue) consists of 9 members of tomato Rab8/RabE and five members of Arabidopsis Rab8/RabE.

Fig. 3.13 shows the Phylogenetic tree of 26 Arabidopsis and 21 Tomato Rab11/RabA GTPase according to the 13th Release of the TIGR DFCI TGI. RabA/Rab11 class is divided into 6 sub-groups. Clade1-(Blue) consists of RabA1 sub group which forms the largest sub group among all the RabA class, wherein eight of tomato RabA1 falls with nine At RabA1. The target gene of our study with TC id TC224574 falls exactly with AtRabA1; again confirming it to be RabA1a. Clade2-(Purple) consists of 3 tomato RabA4 sequences falling in the same cluster with four of At RabA4 sub group. Clade3-(Fuschia) consist of five tomato RabA2 falling in the same cluster of four AtRabA2 sub group with 2 members falling in the RabA2a group and rest three in the AtRabA2b,c and At RabA2d. Clade4-(Blue) consist of one tomato RabA6 in group of two AtRabA6. The last Clade5-(Green) consists of five tomato and five Arabidopsis RabA5 group members.

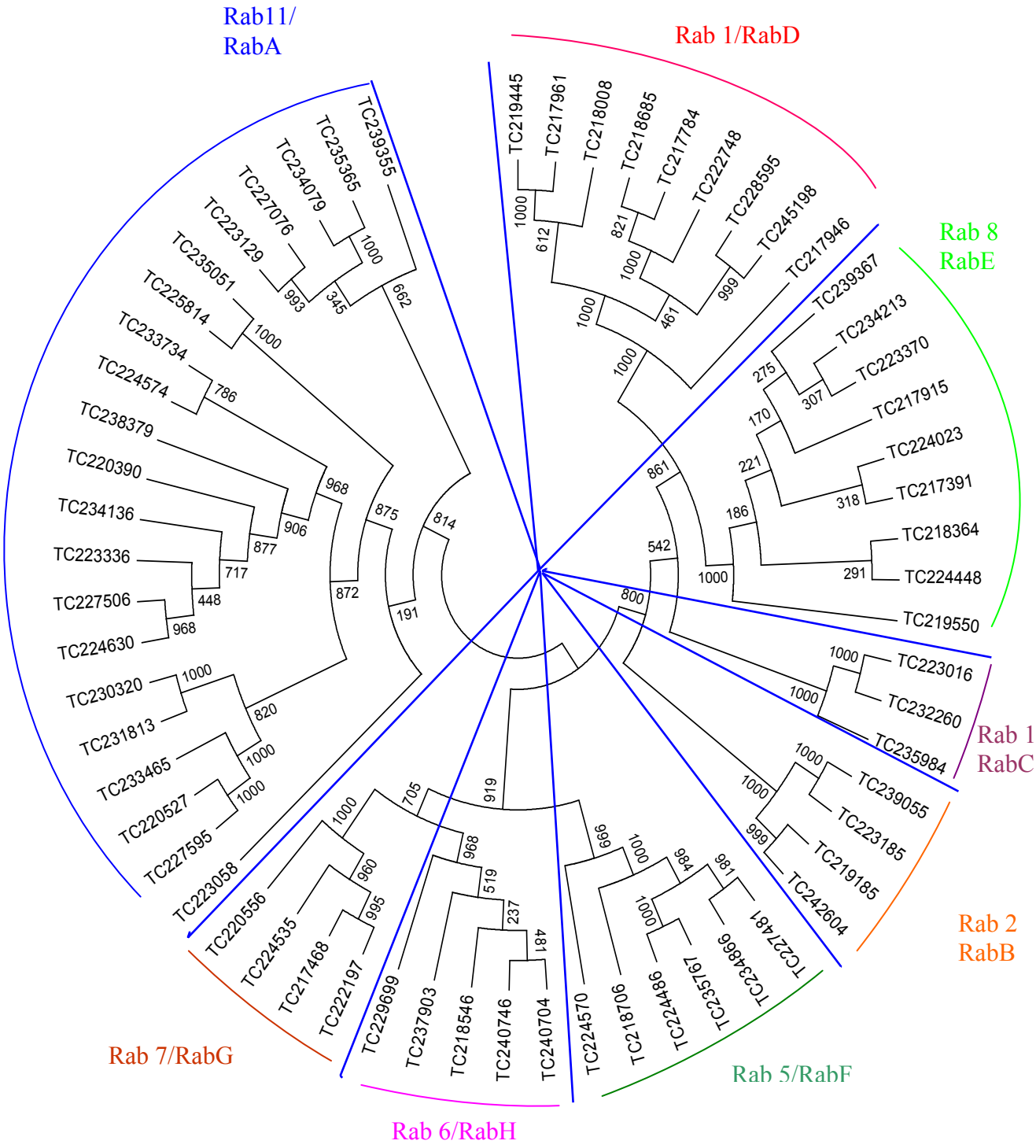


Fig. 3.10 Phylogenetic tree of Rab GTPase in Tomato according to 13th Release of TIGR DFCI TGI.

The figure shows the phylogenetic neighbour joining tree (unrooted) of tomato Rab GTPases with bootstrapped (1000) values. The clear cut distinct classes of Rabs have been highlighted with different colours Rab11 (Blue), Rab1 (Red), Rab8 (Light Green), Rab18 (Magenta), Rab2 (Orange), Rab5 (Green), Rab6 (Pink), and Rab7 (Brown)

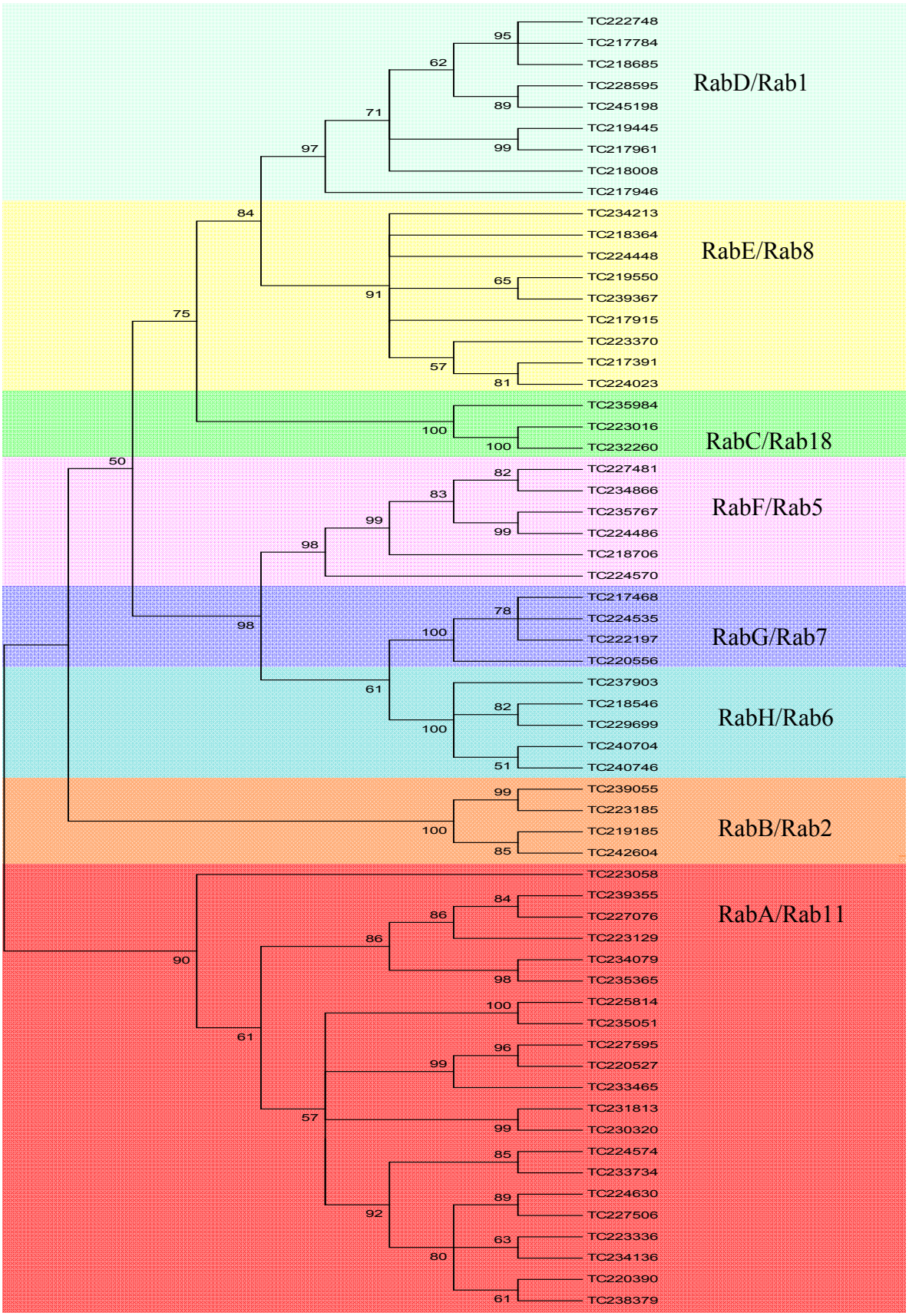


Fig 3.11 Phylogenetic Tree of the latest tomato Rab GTPase according to the 13th release of the TIGR DFCI -TGI. 50% value was set as the cut off for 1000 bootstrap**.** Clear and distinct clades are visible to classify the Rab GTPase with high confidence in their respective groups of Rab1 to Rab18.

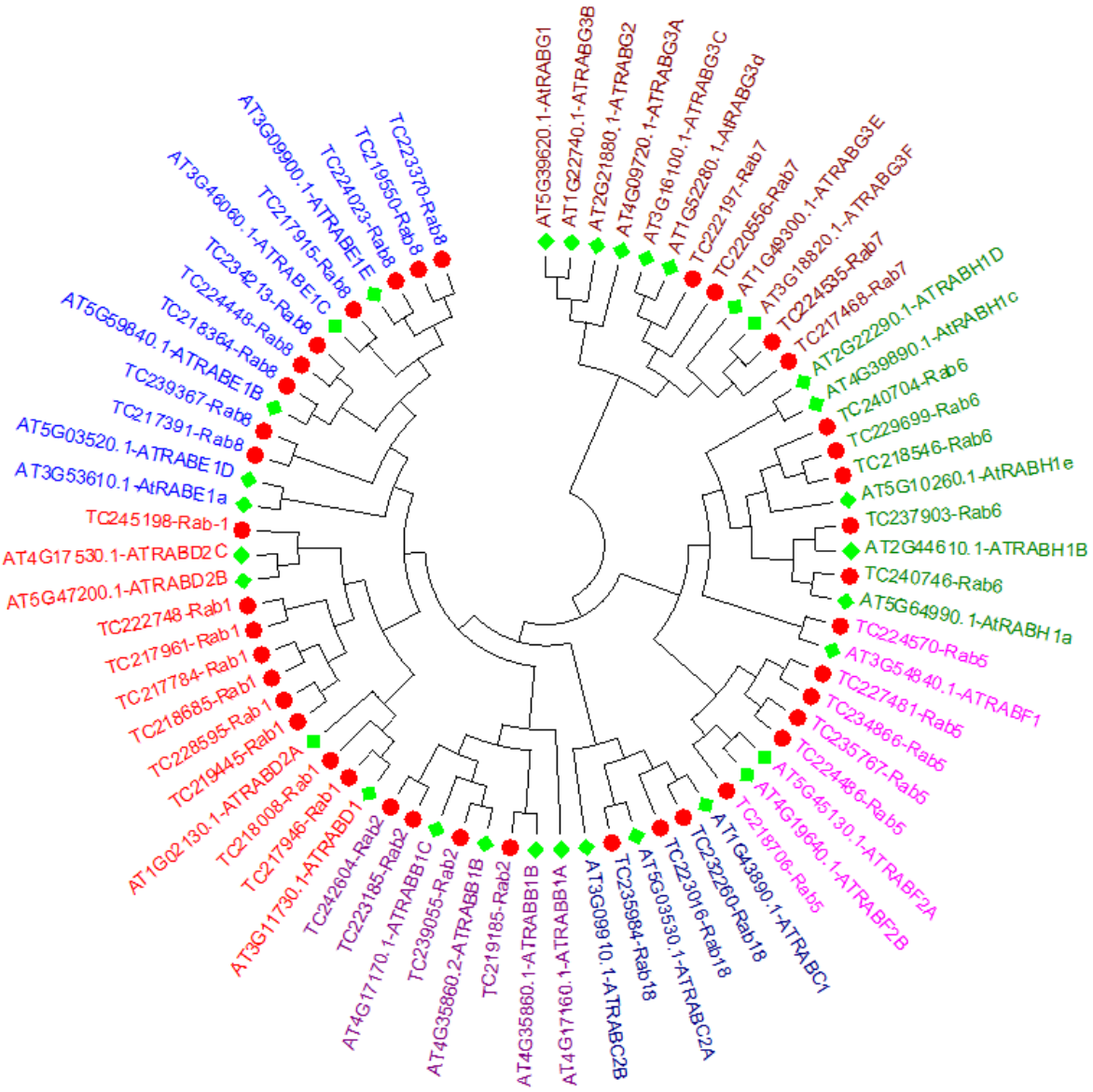


Fig3.12 Phylogenetic tree of Arabidopsis and Tomato Rab1, 2, 5, 6, 7, 8 and Rab18 GTPase according to the 13th Release of the TIGR DFCI TGI

The evolutionary history was inferred using the Minimum Evolution method. The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The evolutionary distances were computed using the Poisson correction method and are in the units of the number of amino acid substitutions per site. The ME tree was searched using the Close-Neighbor-Interchange (CNI) algorithm at a search level of 1. The Neighbor-joining algorithm was used to generate the initial tree. The analysis involved 72 amino acid sequences. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA6. The tree shows different Tomato and Arabidopsis Rab GTPases of different classes ranging from the Rab1, 2, 5, 6, 7, and Rab18.

Node Markers: ● The red filled circle represents tomato sequence Ids.
◆ The light green filled diamond represents the Arabidopsis sequence ids.

Colour codes for branches and taxa: Clade1: Brown (Rab7/RabG), Clade2-Green (Rab6/RabH); Clade3-Fuschia (Rab5/RabF); Clade4-Navy Blue (Rab18/RabC); Clade5-Purple (Rab2/RabB); Clade6-Red (Rab1/RabD); Clade7-Blue (Rab8/RabE).

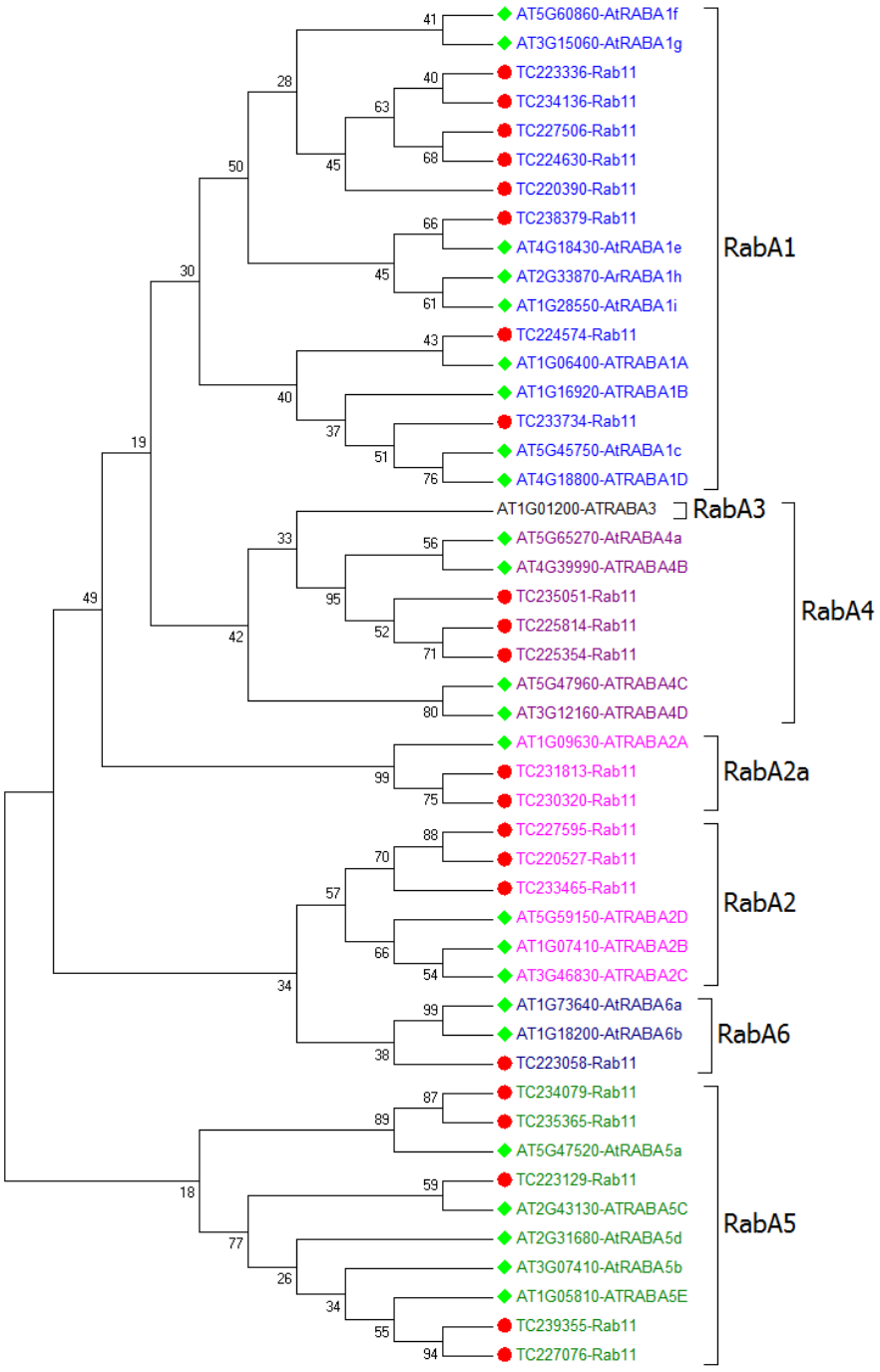


Fig. 3.13 Phylogenetic tree of Arabidopsis and Tomato Rab11/RabA GTPase according to the 13th Release of the TIGR DFCI TGI. The evolutionary history was inferred using the Neighbor-Joining method with 1000 bootstrap. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The evolutionary distances were computed using the Poisson correction method and are in the units of the number of amino acid substitutions per site. The analysis involved 47 amino acid sequences with 26 of AtRabA and 21 of LeRab GTPase. Evolutionary analyses were conducted in MEGA6.

Node Markers: ● The red filled circle represents tomato sequence Ids.

◆ The light green filled diamond represents the Arabidopsis sequence ids.

Colour codes for branches and taxa: Among the RabA/Rab11 sub-groups the following taxon colour code for labels were used: Clade1-Blue-RabA1, Clade2-Purple-RabA4, Clade3-Fuschia-RabA2 (RabA2a and rest of RabA2), Clade4-Blue-RabA6 and Clade5-Green-RabA5.

3.9 Comparison of the TIGR TC, SGN Unigene ids and Probe ids from TED.

Once the identification, classification, sequence alignments and phylogeny of the Rab GTPases from tomato gene index of TIGR were complete, the next step was to identify the Rab GTPases in the SGN database with unique SGN-Unigene ids and in TED database for unique Probe ids.

The comparison of the database was essential in order to find and establish the exact class and number of Rab GTPase in tomato. The advantage in screening the tomato expression database was that once the Probe ids specific to the Rab GTPases are found it can be used to generate the expression profiles for the same in tomato fruit tissues at different developmental stages. Though the difficulty was to establish and ascertain the uniformity among these three different databases. As the scale and time of updates of these databases were different and were not in coherence to the other database. Even if one database was updated and the new IDs were assigned but the corresponding Ids from other database were not checked upon and in most cases were out of date. The timeline of database in case of TIGR was working with the updated database three times in succession e.g. Release 11 to Release 13 of TGI and working through the SGN builds 1 and 2. The entire analysis for each individual step was repeated in order to ensure that the correct set of Rab GTPases were in hand for analysis.

Table 3.14 shows different *Rab* genes and the group of *Rab* genes in tomato. The second and third column shows the TIGR TC accession numbers according to the 12^{th} and 13^{th} release of the database. In case of the 13^{th} release the TIGR TC accession numbers have changed. From each individual Blast results the information regarding complete Uniprot ids were found as shown in 4^{th} column. The Unique SGN ids from build 2 corresponding to the TC numbers from the TIGR database were obtained after exhaustive Blast search. The discrepancy among these databases is evident as they are updated at different times. Hence, it was difficult to come to a concrete conclusion to correlate these databases. But for some of the TC and SGN ids that matched 100%, they were used to extract information from the tomato expression database. A separate database was created in excel to extract the information regarding the expression profile of the specific Rab genes from the DFCI tomato gene index of TIGR database.

Rab GTPases which are expressed in different tissues were counted after mining the information from TIGR TC expression in different tissues of tomato. Particular emphasis was given for those which were highly expressed in fruit or in ripening related manner and were scored accordingly as shown in table 3.15

The following report is an example of the individual BLAST result of TIGR TC to the SGN database for mining the SGN-Unigene Ids.

Blast Report of the Tomato Le Rab11 TIGR TC199565 in SGN Database for Unigene search

Query= TC199565 (218 letters) **Database:** Lycopersicon Combined (Tomato) Unigenes

Sequences producing significant alignments:	**Score (bits)**	**E value**
SGN-U564990 Tomato 200607#2 [18 ESTs aligned] (*GB) gi|6688535|emb|CA...	450	1e-127

>**SGN-U564990** Tomato 200607#2 [18 ESTs aligned] (*GB) gi|6688535|emb|CAB65172.1| (e_value=8e-119) Rab11 GTPase [*Lycopersicon esculentum*]; (*ATH) AT1G06400.1 (e_value=2e-98) | Symbol: None | Ras-related GTP-binding protein (ARA-2), identical to Ras-related protein ARA-2 SP:P28185 from (*Arabidopsis thaliana*) | chr1:1950843-1952726 REVERSE | Aliases: T2D23.10, T2D23_10; (*SWP) sp|Q01111|YPT3_NICPL (e_value=1e-115) Ras-related protein YPT3 OS=*Nicotiana plumbaginifolia* GN=YPT3;

Length = 1,206 Score = 450 bits (1157), **Expect** = 1e-127, **Identities** = 218/218 (100%), **Positives** = 218/218 (100%), **Frame** = +3

```
Query: 1   MAGYRGDDEYDYLFKLVLIGDSGVGKSNLLSRFTKNEFNLESKSTIGVEFATKSLNIDGK 60
           MAGYRGDDEYDYLFKLVLIGDSGVGKSNLLSRFTKNEFNLESKSTIGVEFATKSLNIDGK
Sbjct: 108 MAGYRGDDEYDYLFKLVLIGDSGVGKSNLLSRFTKNEFNLESKSTIGVEFATKSLNIDGK 287

Query: 61  VIKAQIWDTAGQERYRAITSAYYRGAVGALLVYDVTRHVTFENVNRWLKELRDHTDPNIV 120
           VIKAQIWDTAGQERYRAITSAYYRGAVGALLVYDVTRHVTFENVNRWLKELRDHTDPNIV
Sbjct: 288 VIKAQIWDTAGQERYRAITSAYYRGAVGALLVYDVTRHVTFENVNRWLKELRDHTDPNIV 467

Query: 121 VMLLGNKSDLRHLVAVSTEEAKSLAEREALYFMETSALEATNVENAFTEALTQIYRIVSK 180
           VMLLGNKSDLRHLVAVSTEEAKSLAEREALYFMETSALEATNVENAFTEALTQIYRIVSK
Sbjct: 468 VMLLGNKSDLRHLVAVSTEEAKSLAEREALYFMETSALEATNVENAFTEALTQIYRIVSK 647


Query: 181 KAVEAGDEGATSTAPPKGQTINIKDEGSAWKKFGCCSS 218
           KAVEAGDEGATSTAPPKGQTINIKDEGSAWKKFGCCSS
Sbjct: 648 KAVEAGDEGATSTAPPKGQTINIKDEGSAWKKFGCCSS 761
```

Manual Annotations

Based on clone cLED-6-C3, annotated by **Rob Alba**

Putative Homology: "GTP-binding protein, rab11" Annotation Keywords: signal transduction; GTP mediated signal cascade

BLAST Annotations

genbank [blastx] Showing best match of 100 recorded

Match: gi|6688535|emb|CAB65172.1| **score:** 431 **e-value:** 8e-119 **Identity:** 99.54% **Span:** 654bp (54.2%) **Frame:** 3

gi|6688535|emb|CAB65172.1| Rab11 GTPase [Lycopersicon esculentum]

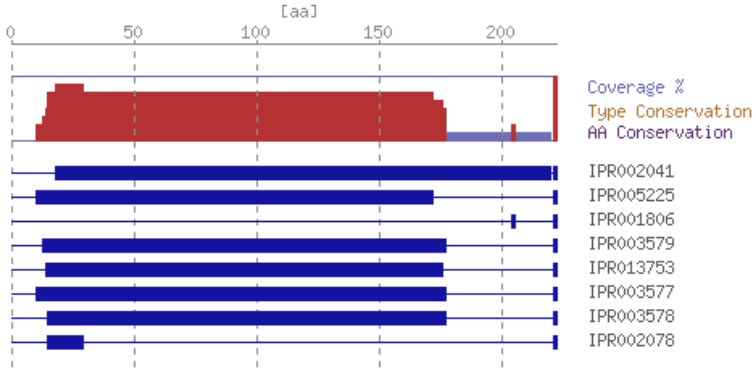
arabidopsis [blastx] Showing best match of 84 recorded

Match: At1G06400.1 **score:** 355 **e-value:** 2e-98 **Identity:** 79.82% **Span:** 654bp (54.2%) **Frame:** 3

AT1G06400.1 | Symbol: None | Ras-related GTP-binding protein (ARA-2), identical to Ras-related pr…

Interpro information for Tomato Rab 11 GTPase

- IPR002041 GTP-binding nuclear protein
- IPR005225 Small GTP-binding protein domain
- IPR001806 Ras GTPase
- IPR003579 **Ras small GTPase, Rab type**
- IPR013753 Ras
- IPR003577 Ras small GTPase, Ras type
- IPR003578 Ras small GTPase, Rho type
- IPR002078 Sigma-54 factor, interaction region



GO Annotations

- GO:0005525 GTP binding
- GO:0006355 regulation of transcription, DNA-dependent
- GO:0005622 intracellular
- GO:0005524 ATP binding
- GO:0003677 DNA binding
- GO:0006886 intracellular protein transport
- GO:0007264 small GTPase mediated signal transduction
- GO:0000160 two-component signal transduction system
- GO:0015031 protein transport
- GO:0008134 transcription factor binding
- GO:0000178 exosome (RNase complex)

Sequences from preceding builds

Unigene	Build
SGN-U319627	Tomato 200607 #1

3.10 Retrieving the Probe ids from Tomato Expression database (TED)

The specific Unigene Ids from SGN database were used to extract the information of individual probe ids from the Tomato Expression Database as shown in Fig 3.15. The probe ids were used in order to find the expression profiles of individual Rab genes available in the TED database during different developmental stages of tomato fruit ripening. These Probe ids were subsequently used to generate the expression pattern of tomato fruits at different developmental stages after anthesis. These probe ids were then used for the hierarchical clustering and K-means clustering to generate the heat map for the expression of genes at different stages.

Table 3.14 Comparison of TIGR TC with SGN-Unigene ids based on Blast searches

Type of Rab	New TC Release 11	New TC Release 12	Uniprot ids	SGN Unigene id
Rab1/RabD	TC170014	TC193212	UniRef100_Q41340	SGN-U319249
Rab1/RabD	TC170107	TC194670	homologue to UniRef100_Q41338	SGN-U315641
		TC193170	UniRef100_Q41338	SGN-U315641
Rab1/RabD	TC170420	TC193154	UniRef100_Q41339	SGN-U316679
Rab1/RabD	TC172978	TC196657	UniRef100_A1E0Y0	SGN-U312492
		TC199235	UniRef100_A1E0Y0	SGN-U312492
Rab1/RabD	TC175258	TC197849	homologue to UniRef100_A1E0Y0	SGN-U312492
Rab1/RabD	TC175806	TC203008	similar to UniRef100_Q40569	SGN-U312490
Rab1/RabD	TC191282	TC217052	*homologue to* UniRef100_Q40569	SGN-U333602
Rab18/RabC	TC178895	TC198096	*homologue to* UniRef100_A7NSN5	SGN-U321767
		TC206013	*homologue to* UniRef100_A7NSN5	SGN-U337452
Rab18/RabC	TC183933	TC210194	*homologue to* UniRef100_A7QJD9	SGN-U571016
Rab2/RabB	TC170492	TC197445	UniRef100_Q946G3	SGN-U562695
Rab2/RabB	TC175074	TC198255	UniRef100_Q946G3	SGN-U562696
Rab2/RabB	TC178011	TC194358	*homologue to* UniRef100_Q9SLP2	SGN-U573044
Rab2/RabB	TC187092	TC194358	*homologue to* UniRef100_Q9SLP2	SGN-U573044
		TC201999	*homologue to* UniRef100_Q9SLP2	SGN-U573044
Rab5/RabF	TC172108	TC193961	*homologue to* UniRef100_Q40570	SGN-U565736
Rab5/RabF	TC177152	TC204357	*homologue to* UniRef100_P29687	SGN-U580477
		TC202099	*homologue to* UniRef100_P31583	SGN-U580477
Rab5/RabF	TC182542	TC209025	*homologue to* UniRef100_P31583	SGN-U578324
Rab5/RabF	TC176115	TC199568	*homologue to* UniRef100_O65842	SGN-U584392

Type of Rab	Old TC Release 11	New TC Release 12	Uniprot ids	SGN Unigene id
Rab6/RabH	TC177750	TC193790	*homologue to* UniRef100_A7R6U5	SGN-U579137
Rab6/RabH	TC183750	TC210040	*homologue to* UniRef100_Q4ABP5	SGN-U579137
Rab6/RabH	TC184604	TC210789	*homologue to* UniRef100_Q40525	SGN-U581580
Rab6/RabH	TC187308	TC213216	*homologue to* UniRef100_A7R6U5	SGN-U580744
Rab6/RabH	TC187358	TC213255	*homologue to* UniRef100_A7R6U5	SGN-U592725
Rab7/RabG	TC171871	TC197334	*homologue to* UniRef100_A0FCQ6	SGN-U578936
Rab7/RabG	TC172410	TC209067	UniRef100_Q38JI9	SGN-U579432
Rab7/RabG	TC174722	TC199544	*homologue to* UniRef100_Q40213	SGN-U579092
Rab7/RabG	TC177559	TC204750	*homologue to* UniRef100_A0FCQ6	SGN-U578936
Rab7/RabG	TC177740	TC206081	*homologue to* UniRef100_Q40528	SGN-U582457
		TC197104	*homologue to* UniRef100_Q40528	SGN-U582457
Rab7/RabG	TC181072	TC194527	UniRef100_Q38JI9	SGN-U579432
		TC209067	UniRef100_Q38JI9	SGN-U579432
Rab8/RabE	TC170347	TC193111	*homologue to* UniRef100_Q8W3J2	SGN-U578937
Rab8/RabE	TC170684	TC193590	UniRef100_Q9SWV8	SGN-U578498
		TC199454	UniRef100_Q39433	SGN-U578498
Rab8/RabE	TC171031	TC192325	UniRef100_Q40177	SGN-U578346
		TC191969	UniRef100_Q40177	SGN-U578346
Rab8/RabE	TC173103	TC193590	UniRef100_Q9SWV8	SGN-U578498
		TC199454	UniRef100_Q39433	SGN-U578498
Rab8/RabE	TC174020	TC194772	*homologue to* UniRef100_Q8W3J3	SGN-U577627
Rab8/RabE	TC175489	NA	NA	NA
Rab8/RabE	TC186042	TC212065	*homologue to* UniRef100_Q8W3J3	SGN-U577627
Rab8/RabE	TC188589	TC193590	UniRef100_Q9SWV8	SGN-U578498
Rab8/RabE	TC190150	TC207687	*homologue to* UniRef100_Q9SWV8	SGN-U578188

Type of Rab	Old TC Release 11	New TC Release 12	Uniprot ids	SGN Unigene id
Rab11/RabA	TC171570	TC202193	*homologue to* UniRef100_Q39434	SGN-U569324
		TC195734	*homologue to* UniRef100_Q39434	SGN-U569324
Rab11/RabA	TC172616	TC193549	*homologue to* UniRef100_A7QPP6	SGN-U577848
		TC207615	*homologue to* UniRef100_A7QPP6	SGN-U577848
Rab11/RabA	TC173506	TC200149	*similar to* UniRef100_A7PH55	SGN-U577717
Rab11/RabA	TC173594	TC200286	*similar to* UniRef100_Q40522	SGN-U569627
Rab11/RabA	TC174729	TC192047	*homologue to* UniRef100_Q40522	SGN-U569627
Rab11/RabA	TC176217	TC199565	UniRef100_Q9SCB1	SGN-U564990
Rab11/RabA	TC176819	TC195610	*homologue to* UniRef100_Q40521	SGN-U564989
Rab11/RabA	TC177424	TC202193	*homologue to* UniRef100_Q39434	SGN-U569324
Rab11/RabA	TC178173	TC198194	*homologue to* UniRef100_Q40524	SGN-U564991
Rab11/RabA	TC180101	TC207026	*homologue to* UniRef100_Q39434	SGN-U569326
Rab11/RabA	TC180423	TC207267	*homologue to* UniRef100_A7QDG9	SGN-U578944
Rab11/RabA	TC180587	TC205638	*homologue to* UniRef100_A7QC87	SGN-U569325
		TC204406	*similar to* UniRef100_A7QC87	SGN-U569325
Rab11/RabA	TC180845	TC207551	*homologue to* UniRef100_Q9SPK2	SGN-U564984
Rab11/RabA	TC181854	TC208438	*homologue to* UniRef100_Q40522	SGN-U569628
Rab11/RabA	TC182133	TC208692	*similar to* UniRef100_Q40200	SGN-U564985
Rab11/RabA	TC182649	TC212051	*similar to* UniRef100_Q39860	SGN-U568799
		TC201753	*similar to* UniRef100_Q40197	SGN-U568799
Rab11/RabA	TC183847	TC211111	*homologue to* UniRef100_A7QPP6	SGN-U577600
		TC202127	*homologue to* UniRef100_Q08149	SGN-U577600
Rab11/RabA	TC185090	TC211213	*similar to* UniRef100_Q08149	SGN-U584956

Table 3.15 Expression of Le Rab GTPase in Fruits from the 12th Release of TIGR DFCI TGI and Probe Ids from TED

Rab1/RabD

TC Release 11	New TC Release 12	Type of Rab	Highest in	Fruits	No.s in fruits	Total	%	Probe ID from TED
TC172978	TC199235	Rab1/RabD	BF	BF; IGF	4	6	66.67	1-1-7.2.14.18
TC175258	TC197849	Rab1/RabD	MGFruit	MGF;RRpF; LeMF; Sl MTF; BF	7	13	53.85	1-1-2.4.18.21
TC191282	TC217052	Rab1/RabD	LeMTF	LeMTF	1	2	50.00	1-1-5.2.17.2
TC172978	TC196657	Rab1/RabD	SlMT fruit	SlMTF; RRpF; BF; MF	9	22	40.91	1-1-2.4.18.21
TC175806	TC203008	Rab1/RabD	SlMTLeaf	BF; LeMF; IGF; MGF;RRpF	15	48	31.25	1-1-5.2.17.2 (3')
TC170107	TC194670	Rab1/RabD	Ovary	IMGF, MTF, RRpF	8	32	25.00	1-1-7.2.14.18
TC170420	TC193154	Rab1/RabD	Sl Leaf	LeMF, BF	4	27	14.81	1-1-7.2.14.18
	TC193170	Rab1/RabD	Root	LeMF	1	9	11.11	1-1-7.2.14.18
TC170014	TC193212	Rab1/RabD	pollen	MGF	1	11	9.09	1-1-7.2.14.18(5')

Rab2/RabB

TC Release 11	New TC Release 12	Type of Rab	Highest in	Fruits	No in fruits	Total	%	Probe ID from TED
TC170492	TC197445	Rab2/RabB	BF	BF; TF; leMF; RTP; IMGF MGF	16	25	60.00	1-1-5.2.14.7(5')
TC187092	TC201999	Rab2/RabB	Fruit	TF	1	2	50.00	1-1-4.4.2.12(3')
TC178011	TC194358	Rab2/RabB	SlMTFruit	MTF; LeMF	7	18	38.89	1-1-4.4.2.12(5')
TC187092	TC194358	Rab2/RabB	SlMTFruit	MTF; LeMF	7	18	38.89	1-1-4.4.2.12(5')
TC175074	TC198255	Rab2/RabB	Sl MT Leaf	LeMF; MGF	2	10	20.00	1-1-6.1.12.13(3')

Rab5/RabF

TC Release 11	New TC Release 12	Type of Rab	Highest in	Fruits	No in fruits	Total	%	Probe ID from TED
TC176115	TC199568	**Rab5/RabF**	Sl MT Leaf	BF;LeMF; LeMTF; SlMTF; TMGF	8	21	38.10	1-1-4.1.18.20(3')
TC182542	TC209025	**Rab5/RabF**	LeMF	LeMF	2	6	33.33	1-1-1.4.13.4(3')
TC177152	TC202099	**Rab5/RabF**	Sl MTLeaf	IMGF; MGF	2	9	22.22	1-1-7.2.18.15(3') 1-1-7.2.18.15(5')
TC172108	TC193961	**Rab5/RabF**	SlMTLeaf	SlMTF; LeMF	5	25	20.00	No hits found
TC177152	TC204357	**Rab5/RabF**	TPR		0	3	0.00	1-1-7.2.18.15(3') 1-1-7.2.18.15(5')

Rab6/RabH

TC Release 11	New TC Release 12	Type of Rab	Highest in	Fruits	No in fruits	Total	%	Probe ID from TED
TC187308	TC213216	**Rab6/RabH**	RTPericarp	RTP; BF	3	4	75.00	1-1-5.1.4.17(5')
TC183750	TC210040	**Rab6/RabH**	IMGF	IMGF; MGF	2	3	66.67	1-1-5.1.4.17(5')
TC177750	TC193790	**Rab6/RabH**	TSC	LeMTF; LEMF; MGF	3	6	50.00	1-1-5.1.4.17(5')
TC187358	TC213255	**Rab6/RabH**	BF	BF	1	2	50.00	1-1-5.1.4.17(5')
TC184604	TC210789	**Rab6/RabH**	LeMF	LEMF; IMGF; LeMTF; TMGF	9	29	31.03	1-1-6.4.18.15(3')

Rab7/RabG

TC Release 11	New TC Release 12	Type of Rab	Highest in	Fruits	No in fruits	Total	%	Probe ID from TED
TC172410	TC209067	Rab7/RabG	LeMF	LeMF; SlMTF;BF; IMGF;MGF;RRF;TF	26	57	45.61	1-1-8.4.11.19(3')
TC181072	TC209067	Rab7/RabG	LeMF	LeMF; SlMTF;BF; IMGF;MGF;RRF;TF	26	57	45.61	1-1-8.4.11.19(3')
TC181072	TC194527	Rab7/RabG	TF	TF;TF;LeMF	5	11	45.45	1-1-8.4.11.19(3')
TC174722	TC199544	Rab7/RabG	TFlower	SlMTF;BF	2	6	33.33	1-1-7.3.17.2(5')
TC177740	TC197104	Rab7/RabG	SlMTLeaf	TMGF	1	5	20.00	1-1-7.3.17.2(5')
TC171871	TC197334	Rab7/RabG	SlMTLeaf	SlMTF	1	14	7.14	1-1-7.3.17.2(5')
TC177559	TC204750	Rab7/RabG	TS/M	--	0	2	0.00	1-1-8.4.11.19(3')
TC177740	TC206081	Rab7/RabG	TPR	--	0	2	0.00	1-1-7.3.17.2(5')

Rab8/RabE

TC Release 11	New TC Release 12	Type of Rab	Highest in	Fruits	No in fruits	Total	%	Probe ID from TED
TC170347	TC193111	Rab8/RabE	TCT	BF; LeMTF;RRF	4	13	30.77	1-1-7.2.18.15(3')
TC171031	TC192325	Rab8/RabE	TTS	TF;LeMTF;BF;LeMF	11	37	29.73	1-1-3.4.14.21(5')
TC174020	TC194772	Rab8/RabE	SlMTFruit	SlMTF;IGF;MGF	5	17	29.41	1-1-5.4.13.14(5')
TC170684	TC193590	Rab8/RabE	Ovary	RTP;BF	2	14	14.29	1-1-3.1.1.3(5')
TC173103	TC193590	Rab8/RabE	Ovary	RTP;BF	2	14	14.29	1-1-3.1.1.3(5')
TC188589	TC193590	Rab8/RabE	Ovary	RTP;BF	2	14	14.29	1-1-3.1.1.3(5')
TC171031	TC191969	Rab8/RabE	TTS	LeMF	1	12	8.33	1-1-3.4.14.21(5')
TC170684	TC199454	Rab8/RabE	TCT	--	0	10	0.00	1-1-3.1.1.3(3')
TC173103	TC199454	Rab8/RabE	TCT	--	0	10	0.00	1-1-3.1.1.3(3')
TC186042	TC212065	Rab8/RabE	TSC	--	0	5	0.00	1-1-1.4.16.10(3') 1-1-5.4.13.14(5')
TC190150	TC207687	Rab8/RabE	SlMTLeaf	--	0	4	0.00	1-1-3.1.1.3(5')

Rab 11

TC Release 11	New TC Release 12	Type of Rab	Highest in	Fruits	No in fruits	Total	%	Probe ID from TED
TC180587	TC204406	**Rab11/RabA**	SlMTF	SlMTF;RTP;MGF	5	7	71.43	1-1-6.4.11.19(3') 1-1-7.1.11.19(5')
TC172616	TC207615	**Rab11/RabA**	LeMFruit	LeMF; SlMTF	2	3	66.67	1-1-3.3.5.9(5')
TC182649	TC201753	**Rab11/RabA**	Root	LeMF;SlMTF;RRF	3	5	60.00	1-1-1.1.9.17(3')
TC173506	TC200149	**Rab11/RabA**	TBFruit	BF;RTP	4	7	57.14	1-1-7.1.8.5(3')
TC176819	TC195610	**Rab11/RabA**	LeMFruit	LeMF;MGF;SlMTF	5	9	55.56	1-1-7.1.8.5(3') 1-1-7.1.8.5(5')
TC172616	TC193549	**Rab11/RabA**	TFruit	TF;LeMF;MTF	6	11	54.55	1-1-3.3.5.9(3')
TC180845	TC207551	**Rab11/RabA**	TBFruit	BF	2	5	40.00	1-1-8.4.11.20(5')
TC182649	TC212051	**Rab11/RabA**	LeMF	LeMF	1	3	33.33	1-1-1.1.9.17(3')
TC181854	TC208438	**Rab11/RabA**	SlLeaf	SlMTF;IGF;LeMF	5	16	31.25	1-1-3.3.5.9(5')
TC178173	TC198194	**Rab11/RabA**	TCT	LeMF;SlMTF;BF	3	11	27.27	1-1-7.4.20.14(5')
TC183847	TC202127	**Rab11/RabA**	SlMTLeaf	SlMTF;IMGF	4	15	26.67	1-1-1.4.6.16(3')
TC176217	TC199565	**Rab11/RabA**	Ovary	SlMTF;TF; RRF	3	14	21.43	1-1-3.3.15.15(3') 1-1-3.3.15.15(5')
TC180587	TC205638	**Rab11/RabA**	TCG	LeMF	1	5	20.00	1-1-7.1.11.19(5')
TC171570	TC202193	**Rab11/RabA**	LeMFruit	LeMF	1	5	20.00	1-1-7.1.11.19(5')
TC177424	TC202193	**Rab11/RabA**	LeMFruit	LeMF	1	5	20.00	1-1-7.1.11.19(5')
TC182133	TC208692	**Rab11/RabA**	SlLeaf	BF	2	11	18.18	1-1-8.4.19.10(5') 1-1-8.4.19.10(3')
TC180423	TC207267	**Rab11/RabA**	LeMFruit	LeMF	2	12	16.67	1-1-3.3.15.15(5')
TC174729	TC192047	**Rab11/RabA**	TTS	TMF;SlMTF	4	25	16.00	1-1-3.3.5.9(5')

Rab11

TC Release 11	New TC Release 12	Type of Rab	Highest in	Fruits	No in fruits	Total	%	Probe ID from TED
TC171570	TC195734	**Rab11/RabA**	TS/M	--	0	8	0.00	1-1-7.1.11.19(5')
TC173594	TC200286	**Rab11/RabA**	TSC	--	0	6	0.00	No hits found
TC180101	TC207026	**Rab11/RabA**	LeLeaf	--	0	3	0.00	1-1-6.4.11.19(3')
TC183847	TC211111	**Rab11/RabA**	TFlower	--	0	3	0.00	1-1-1.4.6.16(3')
TC185090	TC211213	**Rab11/RabA**	Pollen	--	0	8	0.00	1-1-7.1.8.5(3') 1-1-7.1.8.5(5')

Rab18

TC Release 11	New TC Release 12	Type of Rab	Highest in	Fruits	No in fruits	Total	%	Probe ID from TED
TC178895	TC198096	**Rab1/RabD**	SlMTLeaf	SlMTF	2	10	20.00	1-1-5.4.20.14
TC183933	TC210194	**Rab18/RabC**	LeMF	LeMF; TMGF	2	4	50.00	1-1-2.4.9.20(5')
TC178895	TC206013	**Rab1/RabD**	LeMTF	LeMTF; LeMF	2	3	66.67	1-1-5.4.20.14

Abbreviations:

BF: Breaker Fruit; IMGF: Immature Green Fruit; LeL: *Lycopersicum esculentum* Leaf; LeMF: *Lycopersicum esculentum* Maturing Fruit; LeMTF: *Lycopersicum esculentum* MicroTom Fruit; MF: Maturing Fruit; MGF: Mature Green Fruit; RRF: Red Ripening Fruit; RTP: Ripe tomato Pericarp; SlMTF: *Solanum lycopersicum* Micro Tom Fruit

SlMTL: *Solanum lycopersicum* Micro Tom Leaf; TCG: Tomato Crown Gall; TCT: Tomato Callus Tissue; TF: Tomato Fruit; TFL: Tomato Flower Library; TPR: tomato Pseudomonous Resistant; TS/M: Tomato Shoot/Meristem; TSC: Tomato Suspension Culture; TTS: Tomato Transposon Sequencing.

3.11 Hierarchical clustering of the Rab genes for gene expression profiling

The probe ids were used to generate the expression profiles available in the Tomato expression database (TED) database. The tomato expression database contains annotation and sequence information of all probes on the tomato TOM1 cDNA array and TOM2 oligo array and affymetrix genome array. It provides tools for single or batch query for the retrieval of desired information of the probe ids. The individual probe ids were screened with TOM1 cDNA array in the TED database. The hierarchical clustering of all the available probe ids corresponding to Rab GTPase according to the SGN unigene ids (second build) was done. The results are shown in the following pages. While clustering it was found that the expression profile was not available in the database for certain probe ids. The cluster diagram shows the specific Rab GTPase and their relative expression from 7 days after pollination (DAP) upto 57 days after pollination. The figure shows the relative expression at 7, 17, 27, 39, 41, 42, 43, 47, 52, 57 DAP. The green colour shows the down-regulated genes and red shows the up regulated genes whereas black shows no expression at all. Since the amount of information that is available is limited for the expression of all the Rab genes in tomato, in order to come up with the real expression profile of the genes, the candidate genes which are expressed in ripening related manner in tomato fruits a relative expression profile needs to be generated by qRT-PCR at different developmental stages. The expression profiles available in TED can give a fair estimate of *Rab* genes which are expressed and are either down or up regulated during the peak period of ripening.

After getting the expression profiles of the available genes, the cluster diagram was generated for those *Rab* genes for which there was a 100% match between the TIGR TC numbers and SGN unigene ids. This is to ensure that there is no difference between the sequences in two different databases so that the expression profile obtained by the probe id is the real expression profile of that particular gene.

After getting the cluster diagram the objective was to find the individual expression profile of the genes. For this the relative expression data retrieval (TOM1 array) was done for the genes expressed during tomato fruit development and ripening for the wild type tomato plants.

The specific unigene id of *Rab11a* gene was blasted into the TED with TOM1 array (TOM1 cDNA array). Figure 3.14 shows the relative expression profiles of the selected Probe ids in wild type (red), *Nr* mutant (green), *rin* (blue), *nor* (yellow) and *hp* (pink). The relative expression indicates that the expression of *LeRab11* is quiet low in wild type fruits but the expression goes up during 20 to 40 DAP in case of Never ripe (Nr mutants). Thus, the genes can act as the putative candidate genes for further studies as supported by Lu *et al*., (2001).

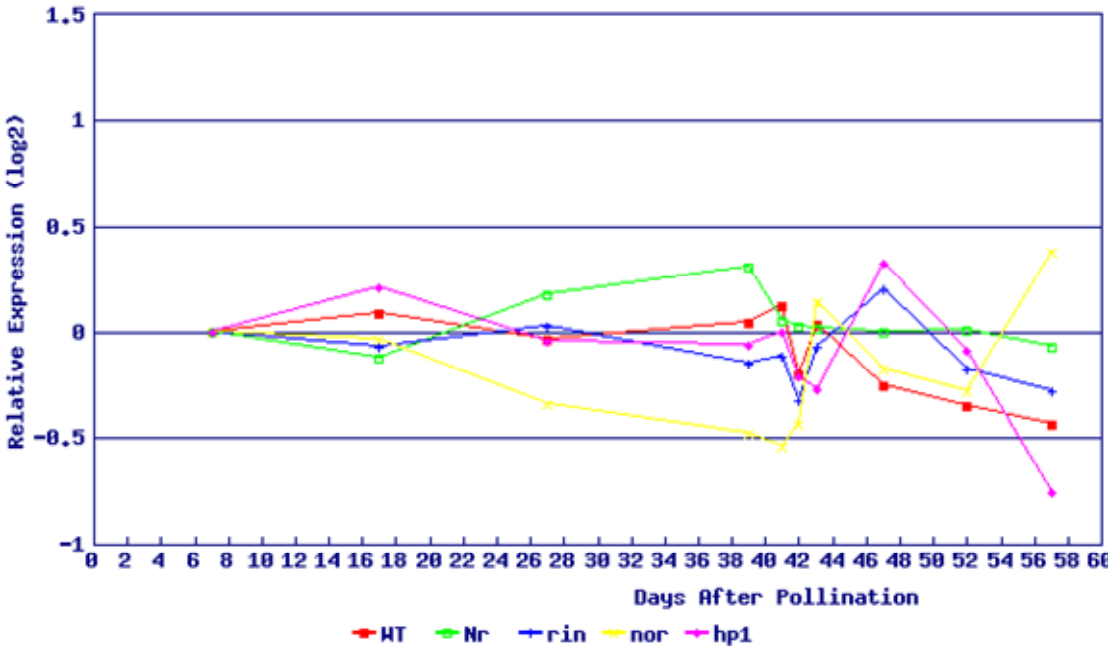


Fig. 3.14 The Relative expression profile of LeRab11a from the TED database. Expression pattern of different mutants and wild type tomato is shown at different developmental stages of fruit ripening. Wild type (Red), *Nr* (Green), *Rin* (Blue), *Nor*(Yellow) and *hp1* (Pink)

The blast results of *LeRab11a* screened with TOM 2 oligo array indicate that most of the probe ids which show the nearest hits are in Arabidopsis, Lotus and cotton. The list of probe ids were generated according to the homology and the nearest match based on the sequence similarity. Although the Boyce Thomson institute has stopped printing the TOM2 oligo arrays but the information might be useful for anticipating the expression patterns of Rab GTPases.

The relative expression profiles obtained from the probe ids used to generate the heat maps for the individual group of Rab GTPases is shown in Appendix section. In case of RabD/Rab1 the corresponding probe id 1-1-5.4.20.14 of TC198096 is up regulated during most of the 17 to 57 DAP. RabB with TC197445 with probe id 1-1-5.2.14.7 is up regulated from 17 to 57 DAP whereas the TC198255 goes up from 27 to 57DAP. In case of Rab5 most of the genes are up regulated at the onset of 57DAP.

Likewise in case of Rab6 most of the genes are up regulated during the mature green stage but the expression goes down during later ripening stages. The most remarkable of all Rab GTPases in tomato is RabG/7 as it is the only case wherein the expression is consistently high during fruit ripening. It can be a suitable candidate gene to study the effects of knockout of this clade of Rab genes. In case of RabE/8 the TC199454 with the corresponding probe id is up regulated during 42 and 47 DAP but the expression goes down later. In case of our target Rab11a (TC199565 with probe id 1-1-3.3.15.15) expression goes down during the later stages of ripening but the gene is up regulated during the initial stages of ripening. Another Rab11 GTPase with id TC211213 having probe id 1-1-7.1.8.5 is down regulated from 17-57 DAP. The Fig. 3.11 shows the top most up regulated Rab GTPase of among all group of Rab GTPase represented with their probe ids.

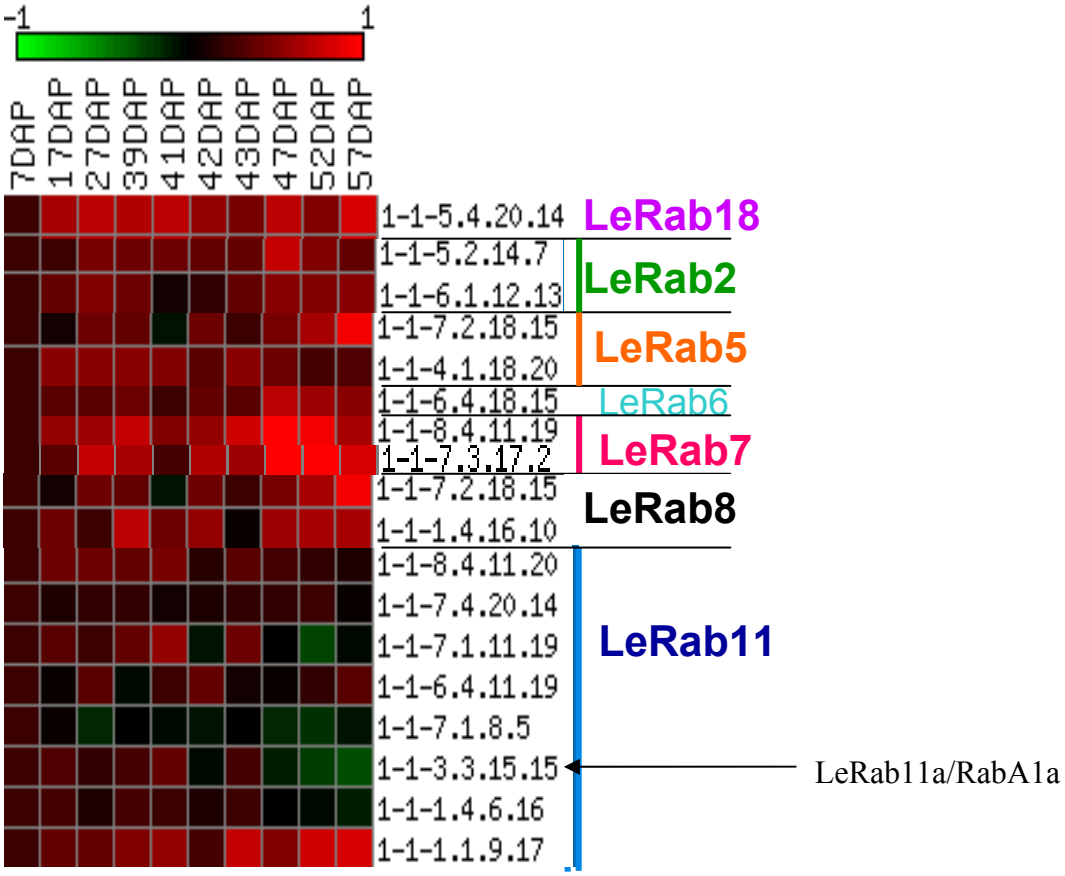


Fig.3.15 Heat map of Tomato Rab GTPases.
Heat map was generated from the best hits of the genes which are up regulated during the different stages of fruit ripening. The up regulated gene were screened and grouped by K-Mean Clustering of all the highly expressed *Rab* genes of all groups during fruit ripening. The *LeRab11a* with probe id 1-1-3.3.15.15 was up regulated from 7 to 41 DPA (Upto Mature green Stage) and was down regulated during 52 and 57 DPA.

3.12 *in silico* analysis of other Small GTPases and regulators of Rab GTPase.

3.12.1 Arf GTPase

ADP-ribosylation factor (ARF) was first identified in case of cholera toxin catalysed by ARF GAP of adenylate cyclase. Mammalian ARFs are grouped into 3 classes according to the size; amino acid sequences and their structure, with ARF1, 2 and 3 in class-I, ARF4 and 5 in class-II and ARF6 in class-III. ARF GTPases are involved in the regulation of the lipid metabolism, vesicle trafficking and interacting with other GTP binding proteins (Boman and Kahn, 1995). ARF recruits COP-I to Golgi for sorting of cargo proteins into the vesicles (Beck *et al*., 2009).

The ARF family is divided into three groups ARF, ARL and SAR. There are 12 ARF GTPase in Arabidopsis (Vernoud *et al.*, 2003) and 14 in Barley. ARFs are usually located on the membranes where the vesicles are formed. Bohelenius *et al.,* in 2010 reported that a GFP tagged to barley ARF was located at the MVB in onion's epidermal cells. It was also reported that ARFB1A was localized at PM whereas ARFA1C was localized at Golgi and endocytic organelles. Inactivation of ARFA1B eliminates ROR2 syntaxin and callose accumulation at the site of attack of the bacteria.

From the TAIR database, a total of 12 ARF GTPase sequences (Table3.16) were retrieved and used for BLAST search in TGI, as a result 13 ARF GTPase were found in tomato. Fig. 3.16 shows the MSA and Fig. 3.17 shows the phylogenetic tree of Arabidopsis and tomato ARF GTPase.

Table 3.16 ARF GTPase in Arabidopsis

ARF Class	Sub Group	Accession Number
ARF A	ATARFA1A	AT1G23490
	ATARFA1B	AT5G14670
	ATARFA1C	AT2G47170
	ATARFA1D	AT1G70490
	ATARFA1E	AT3G62290
	ATARFA1F	AT1G10630
ARF B	ATARFB1A	AT2G15310
	ATARFB1B	AT5G17060
	ATARFB1C	AT3G03120
ARF C	ATARFC1	AT3G22950
ARF D	ATARFD1A	AT1G02440
	ATARFD1B	AT1G02430

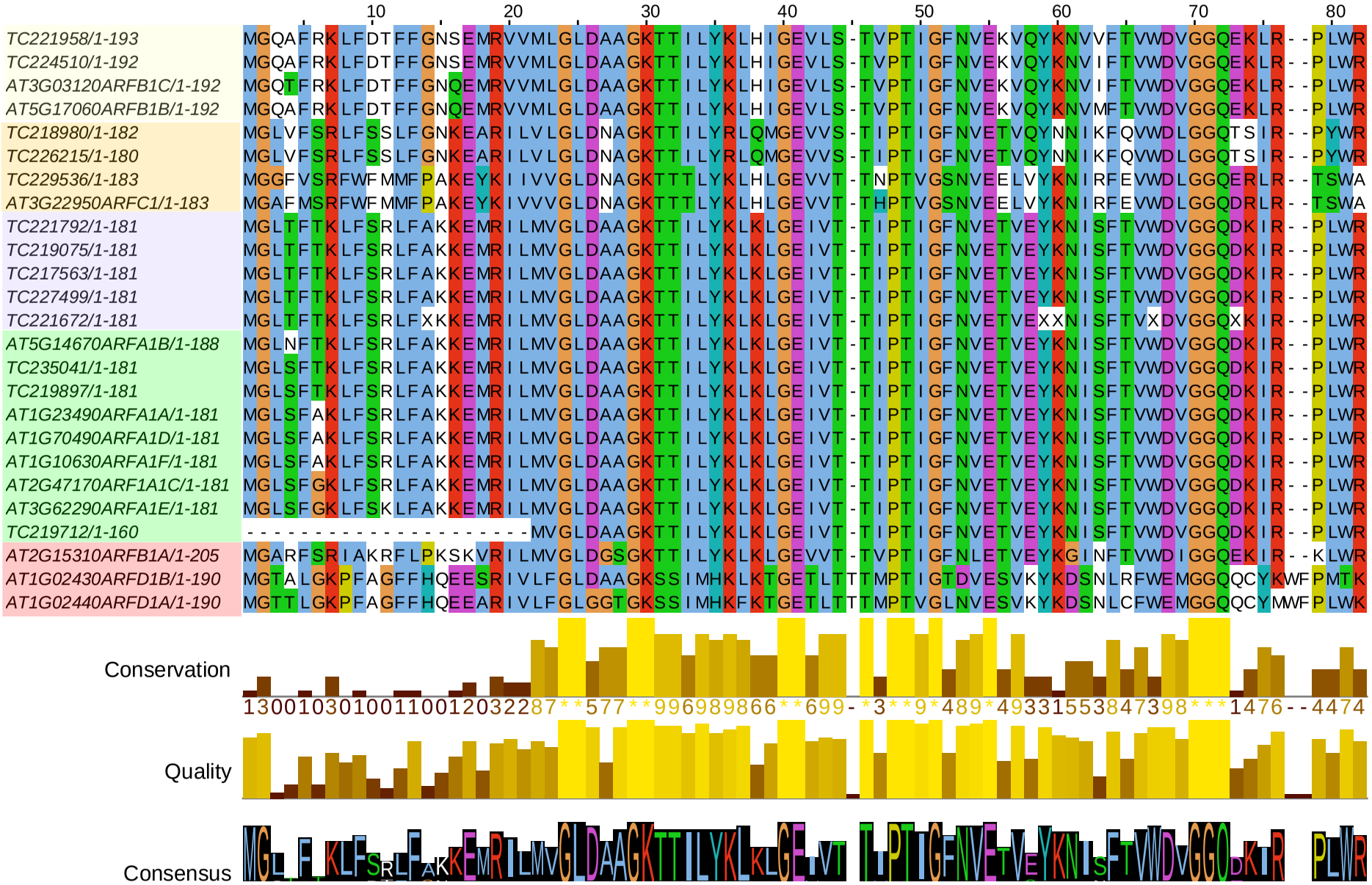


Fig. 3.16 Multiple sequence alignment of Arabidopsis and Tomato ARF GTPase

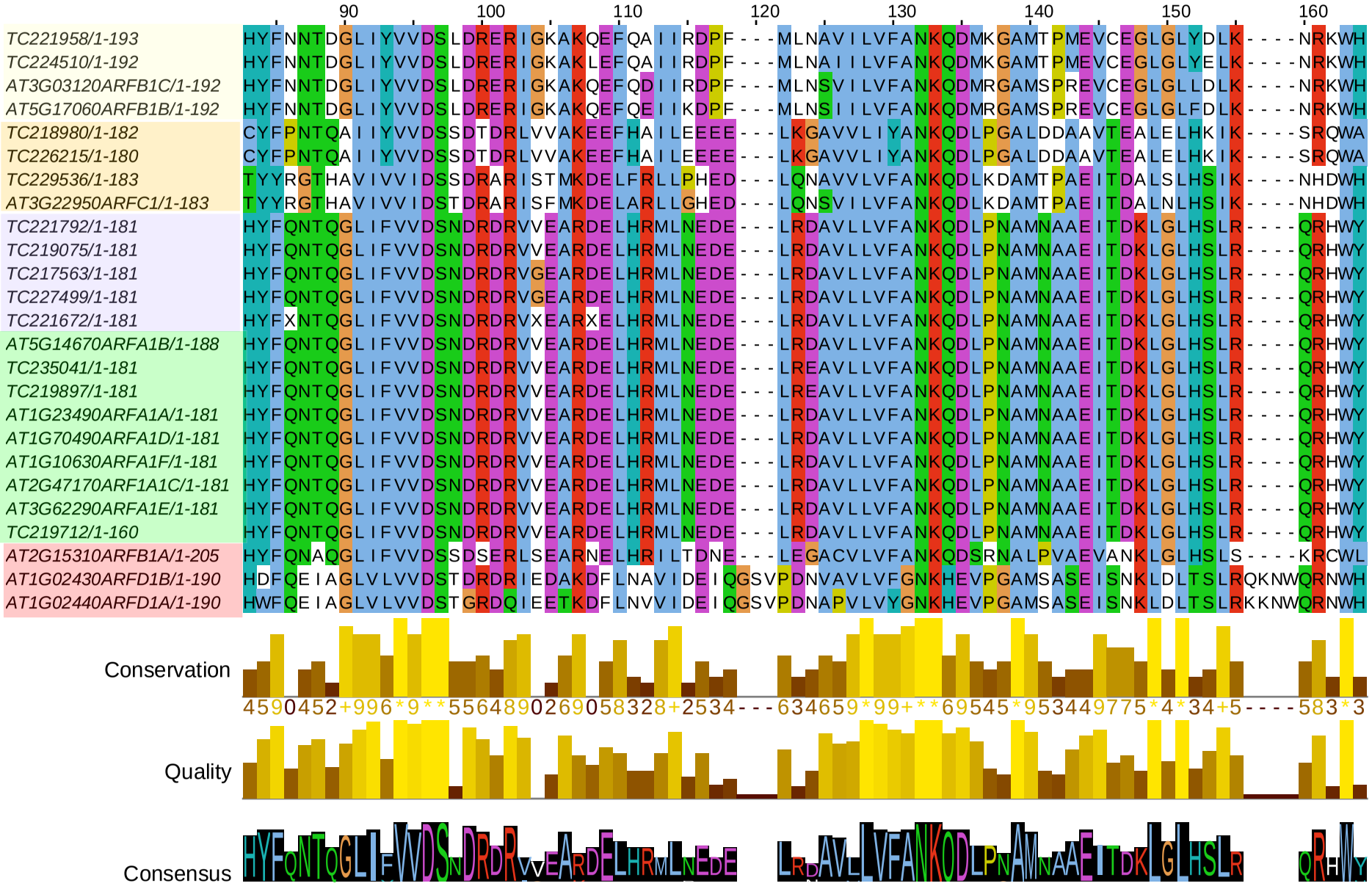


Fig. 3.16 Multiple sequence alignment of Arabidopsis and Tomato ARF GTPase

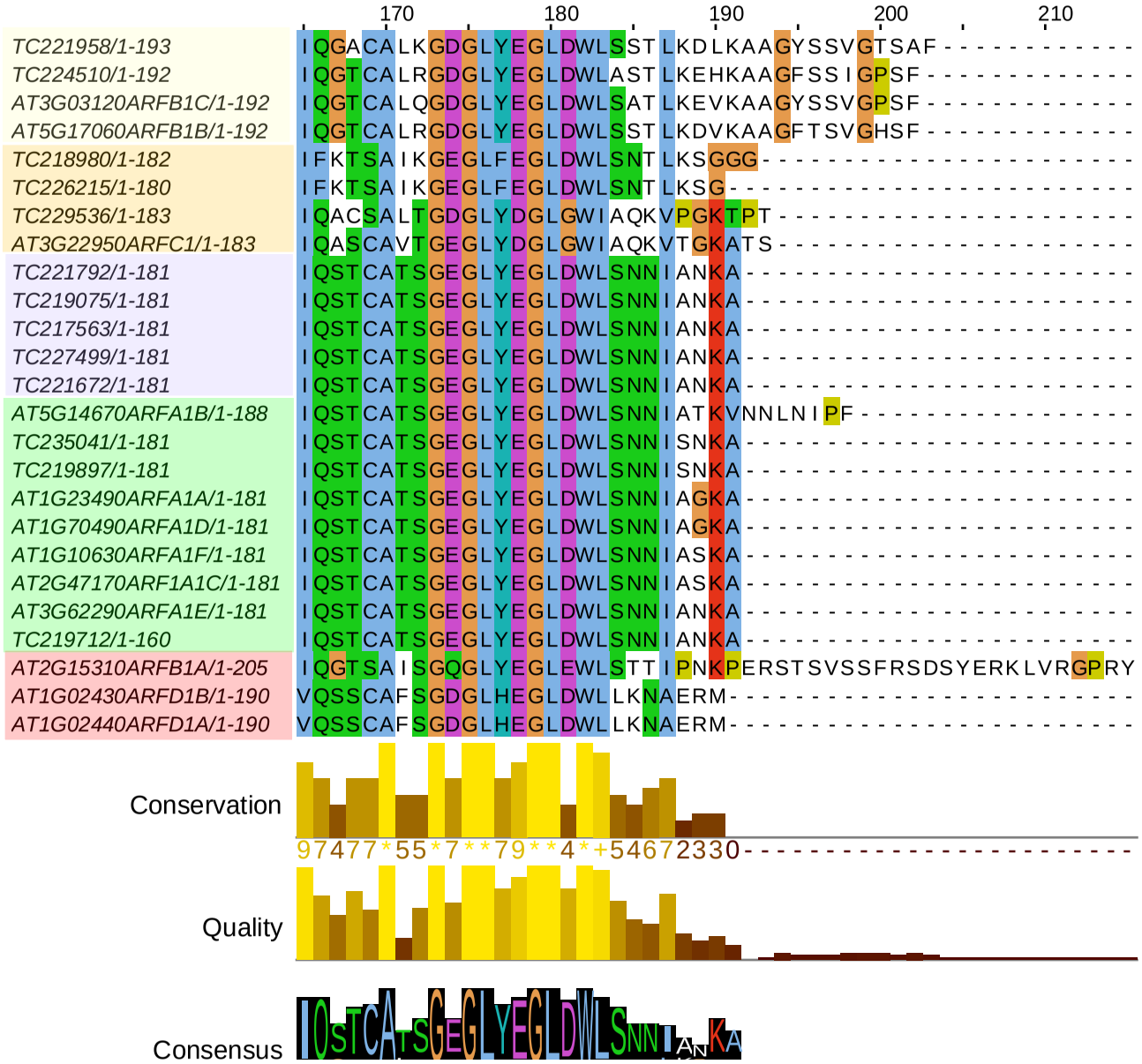


Fig. 3.16 Multiple sequence alignment of Arabidopsis and Tomato ARF GTPase. The MSA was generated by Jalview ver.2.8.2. The conservation histogram and consensus logo of ARF GTPase are shown

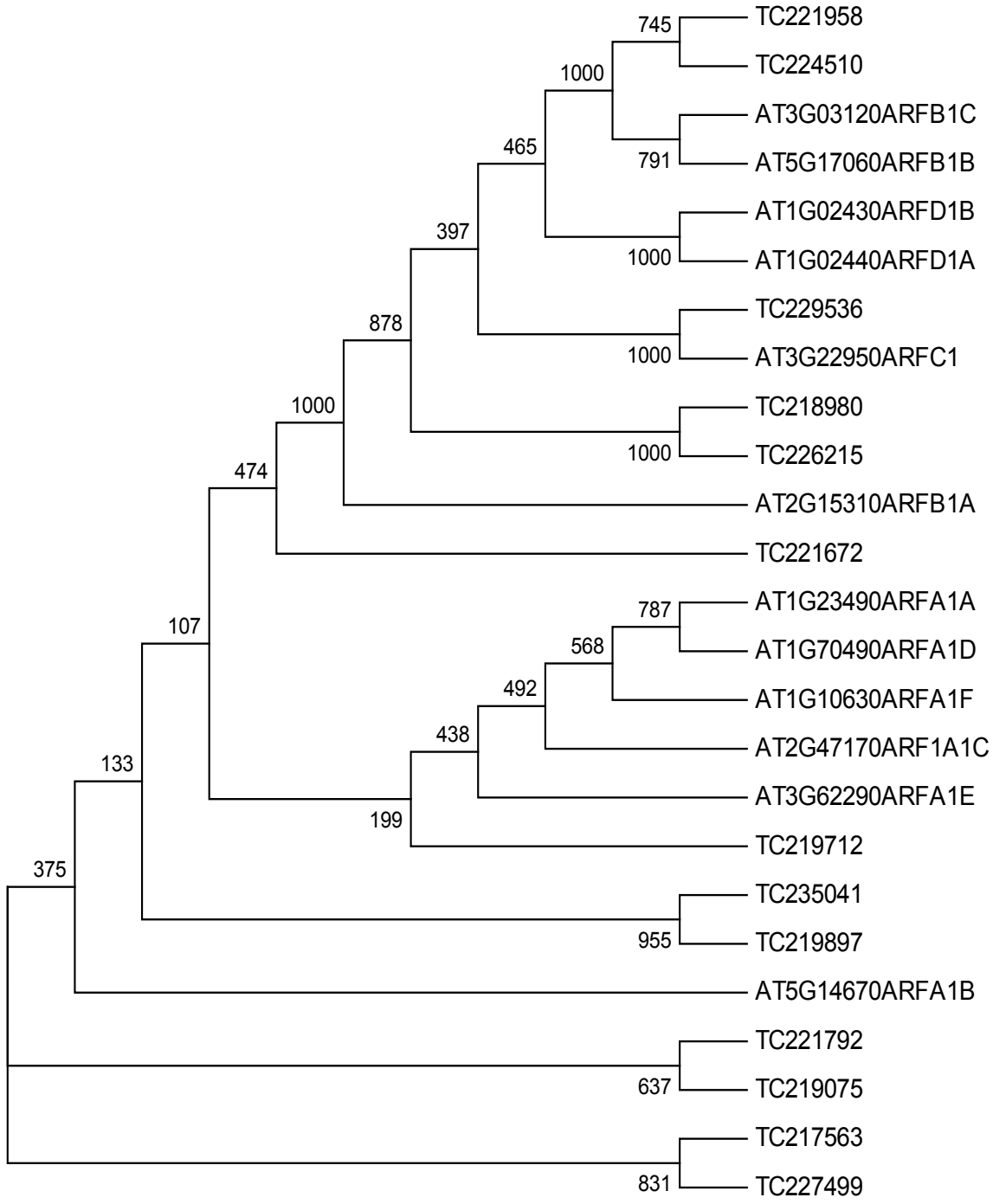


Fig. 3.17 Phylogenetic tree of Arabidopsis and Tomato ARF GTPase

Neighbour joining (NJ) tree of Le vs At ARF GTPases is shown with 1000 boot strap values. TC221958 and TC 224510 fall along with At ARFB, TC229536 falls with ARFC. ARF A constitutes the largest group having highest number of ARF GTPases. TC219712, TC235041, TC219897 lie with AtARFA group. Thus, out of 13 tomato ARF GTPases 9 ARFs fall in the clade with Arabidopsis ARFs. Future release will shed more light on the exact number of ARF GTPases.

3.12.2 ARL GTPase

In plants ARLs are still not fully characterized and well studied. ARF like GTPase (ARL GTPase) belong to the ARF family of GTPases but are more diverse in structure and function than ARFs. ARL1 and ARF related proteins are located at TGN to facilitate vesicle transport at endosomes. ARL2 regulates the folding of β-tubulin. Mutation in ARL2 results in the loss of microtubule function required for cytokinesis. ARL4 and ARL5 proteins are localized to the nucleus and nucleoli (Jacobs *et al.,* 1999). There are 6 ARL GTPase in Arabidopsis, using these as reference a total of 18 TC sequences were found, out of these 5 closely related ARL GTPases can be ascribed to the group in tomato. TC220173, TC227241, TC218063 lie with AtARLA1A clade. TC220753 lie with AtARLC1 and TC219832 falls with AtARLB1. These 5 tomato ARLs fall with At ARLs. Rest of the ARL or ARL like GTPase form a distinct cluster. More information is anticipated in the future releases. Fig 3.18 shows the phylogenetic tree and Fig. 3.19 shows the MSA of Arabidopsis and tomato ARLs.

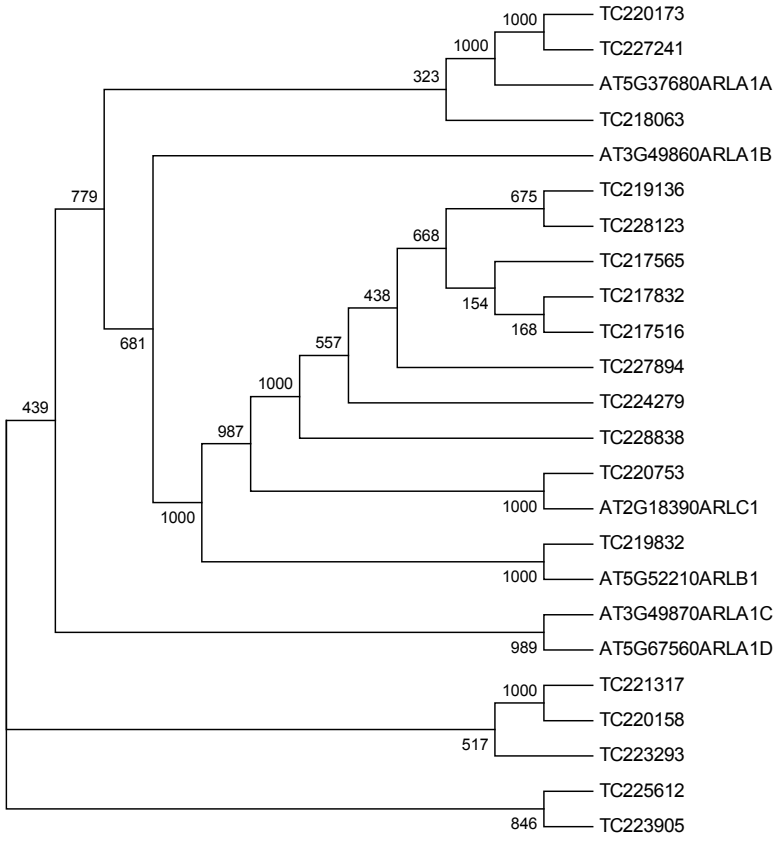


Fig. 3.18 Phylogenetic tree of Arabidopsis and Tomato ARL GTPase: The neighbour joining phylogenetic tree with 1000 bootstrap values is shown. Some of the tomato ARL form a distinct clade but have unique motifs and more likely to be ARL related or ARL-like new parallel group. TC220173, TC227241, TC218063, TC220753 and TC219832 are tomato ARLs.

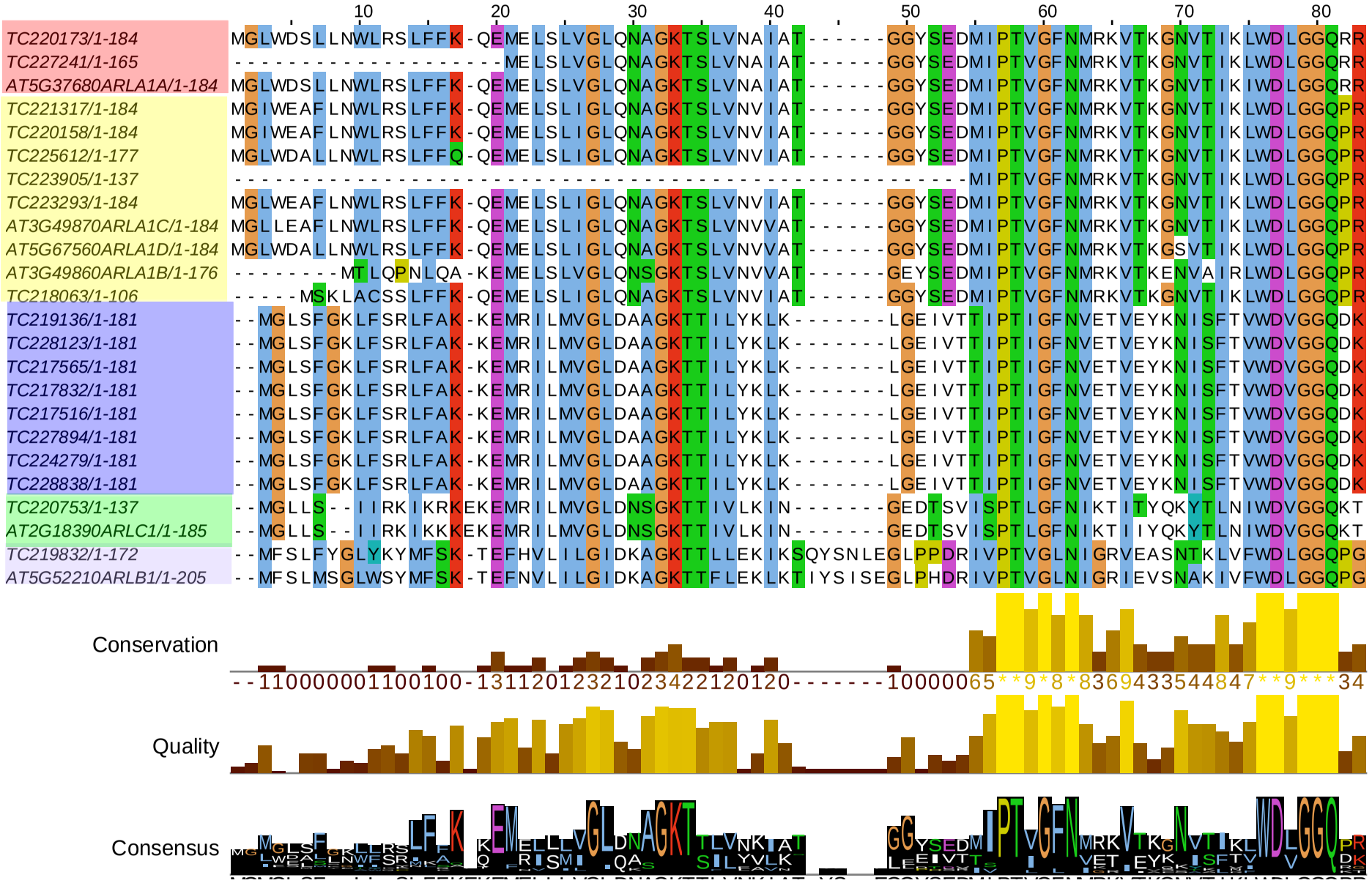


Fig. 3.19 Multiple sequence alignment of Arabidopsis and Tomato ARL GTPase. The MSA was generated by Jalview ver.2.8.2. The conservation histogram and consensus logo of ARL GTPase are shown

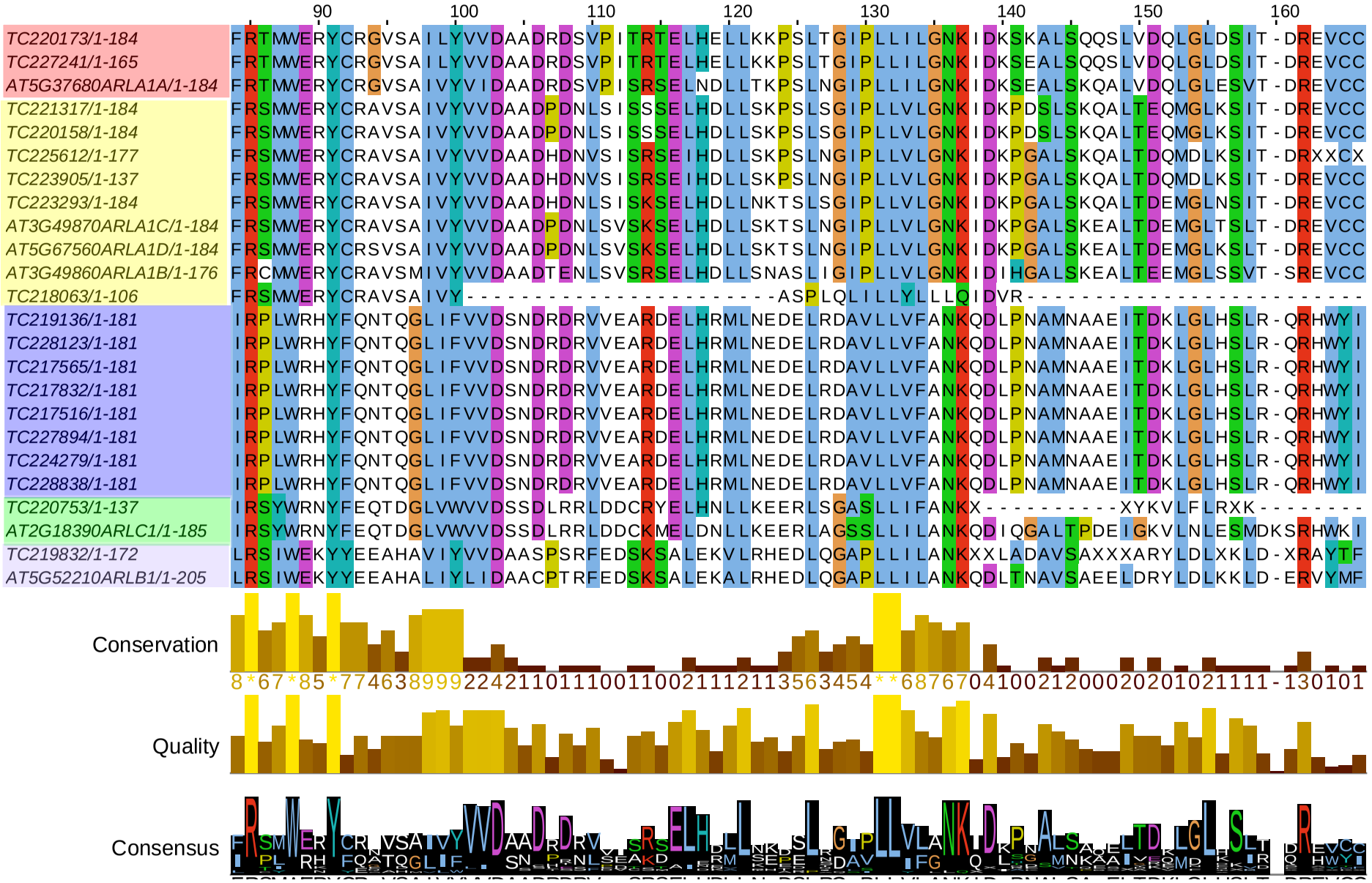


Fig. 3.19 Multiple sequence alignment of Arabidopsis and Tomato ARL GTPase contt...

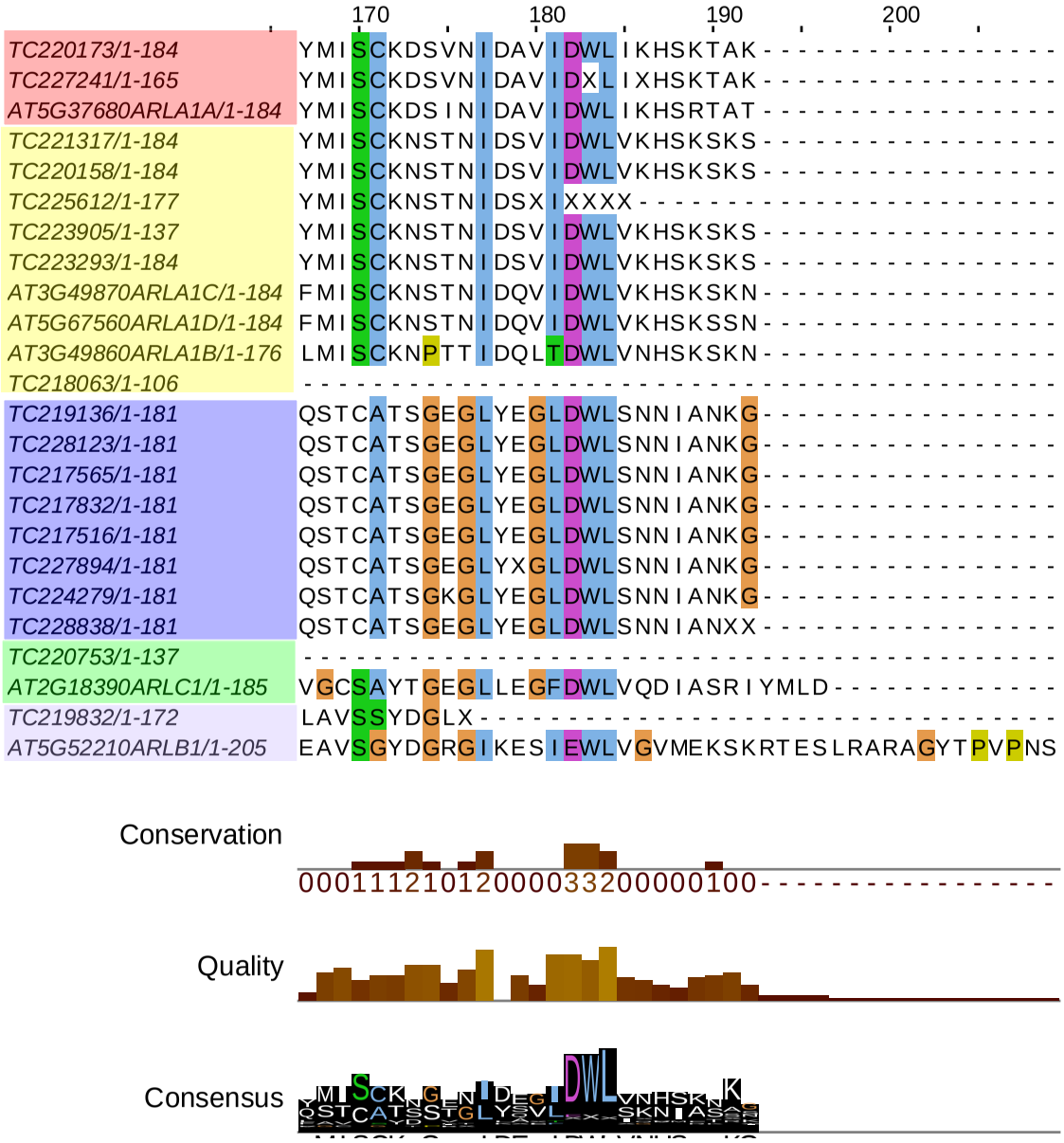


Fig. 3.19 Multiple sequence alignment of Arabidopsis and Tomato ARL GTPase contt...

3.12.3 SAR GTPase

SAR GTPase stands for secretion associated and Ras related GTPase which are responsible for the regulation of anterograde transport from ER to Golgi (Nakano *et al*., 1988)

SAR GTPase belongs to the ARF GTPase family because of an amino terminal amphipathic helix, and have similar function to ARF and are involved in the recruitment of the coat complex during the process of vesicle budding. Sar1 GTPase recruits COPII coat to ER membrane. ARF1 and SAR1 are more abundant in shoots.

There are 3 SAR GTPase in Arabidopsis, after the Blast search in TGI seven SAR and SAR like GTPase were found in tomato as shown in fig 3.20 most of them are quite distinct and does not fall within the same clusters except TC224702 and TC221450 which are more closely related to the Arabidopsis SAR GTPases. It would be interesting to see if the same pattern of these sequences could be observed in the future release of tomato whole genome sequence. That can clearly validate the total number of SAR GTPase present in tomato. Fig.3.21 shows the MSA of At vs Sl SAR GTPase.

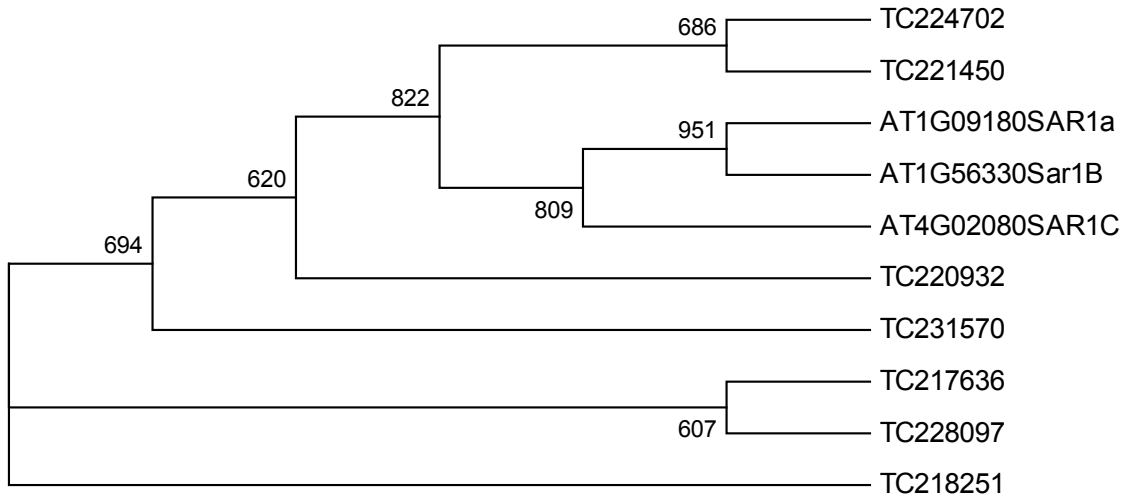


Fig 3.20 Phylogenetic tree of Arabidopsis and Tomato SAR GTPase according to the 13th release of TIGR DFCI TGI.

The neighbour joining phylogenetic tree of Tomato and Arabidopsis SAR GTPase is shown with 1000 bootstrap values. The outlier groups seems to be out of place but sequences have been checked and have been well annotated and seems to fall in the group of SAR GTPase or SAR like GTPases. More information is anticipated in the future releases in order to validate and finalise the exact number of SAR GTPases in tomato and to discard the false positives if any.

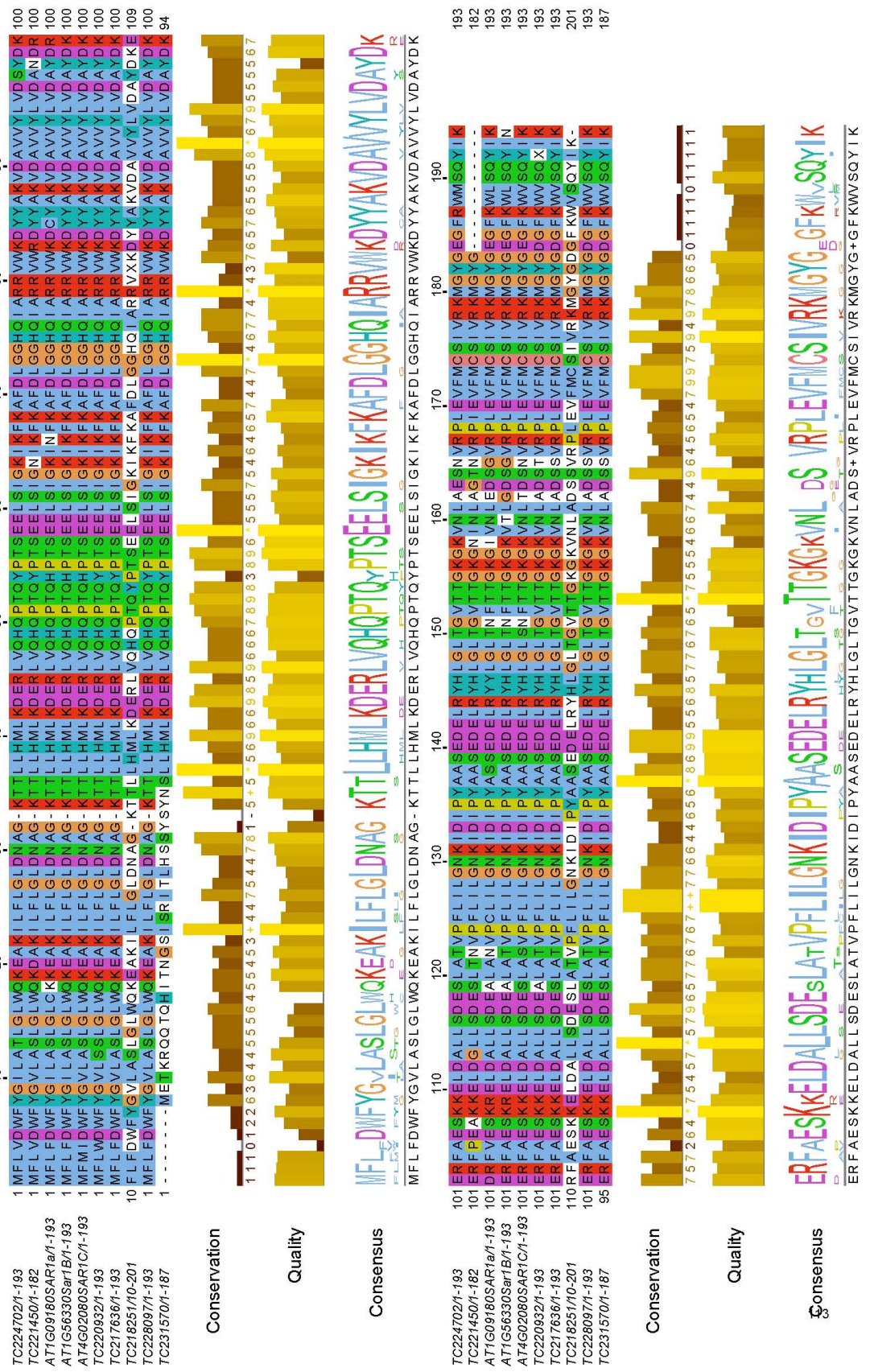


Fig 3.21 MSA of Arabidopsis and Tomato SAR GTPase according to the 13th release of TIGR DFCI TGI.

The MSA of Arabidopsis and Tomtao SAR GTPase was generated by Jalview ver.2.8.2. The conservation plot, histogram and consensus logo of SAR GTPase are shown

3.12.4 ROP GTPase

Rho like GTPase of plants (ROP GTPase), ROP GTPase are sometimes also referred to as RAC because of sharing higher amino acid identity. ROPs are involved in signalling pathway for the regulation of the cytoskeletal organization, vesicular trafficking, cellular polarization for polar growth and cell morphogenesis in plants. There are 11 ROP GTPase found in Arabidopsis. AtRop2 and AtRop4 are confined to the tips of elongating root hairs. Over expression of these genes in Arabidopsis resulted in a phenotype with strong root hairs, including isotropic growth or increased length of root hairs. AtROP also controls apical growth during root hair development (Molendijk *et al.* 2001; Jones *et al.* 2002). In case of tomato only 8 ROP GTPases were found. Fig.3.22 shows the phylogenetic tree comparing the Arabidopsis and tomato ROP GTPases. Fig 3.23 shows the MSA of At and Sl ROP GTPase.

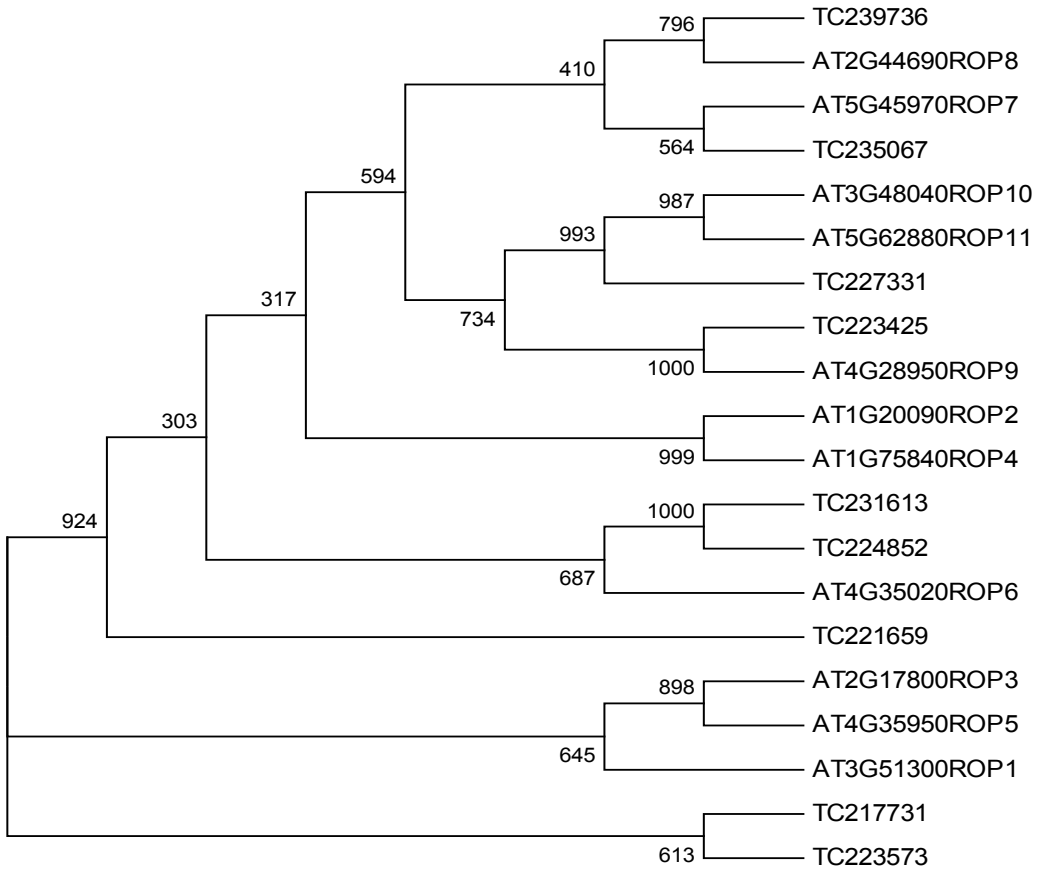
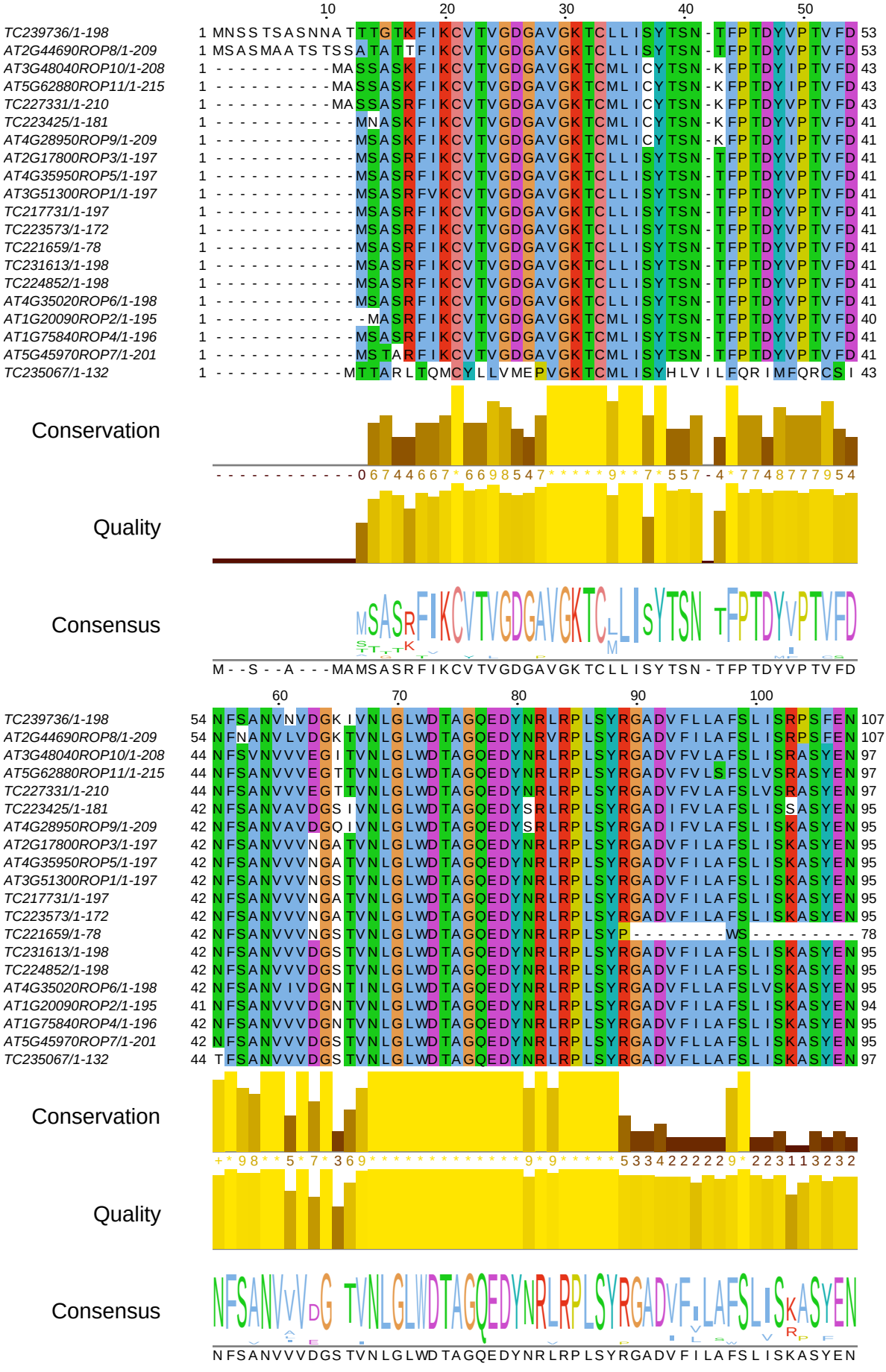
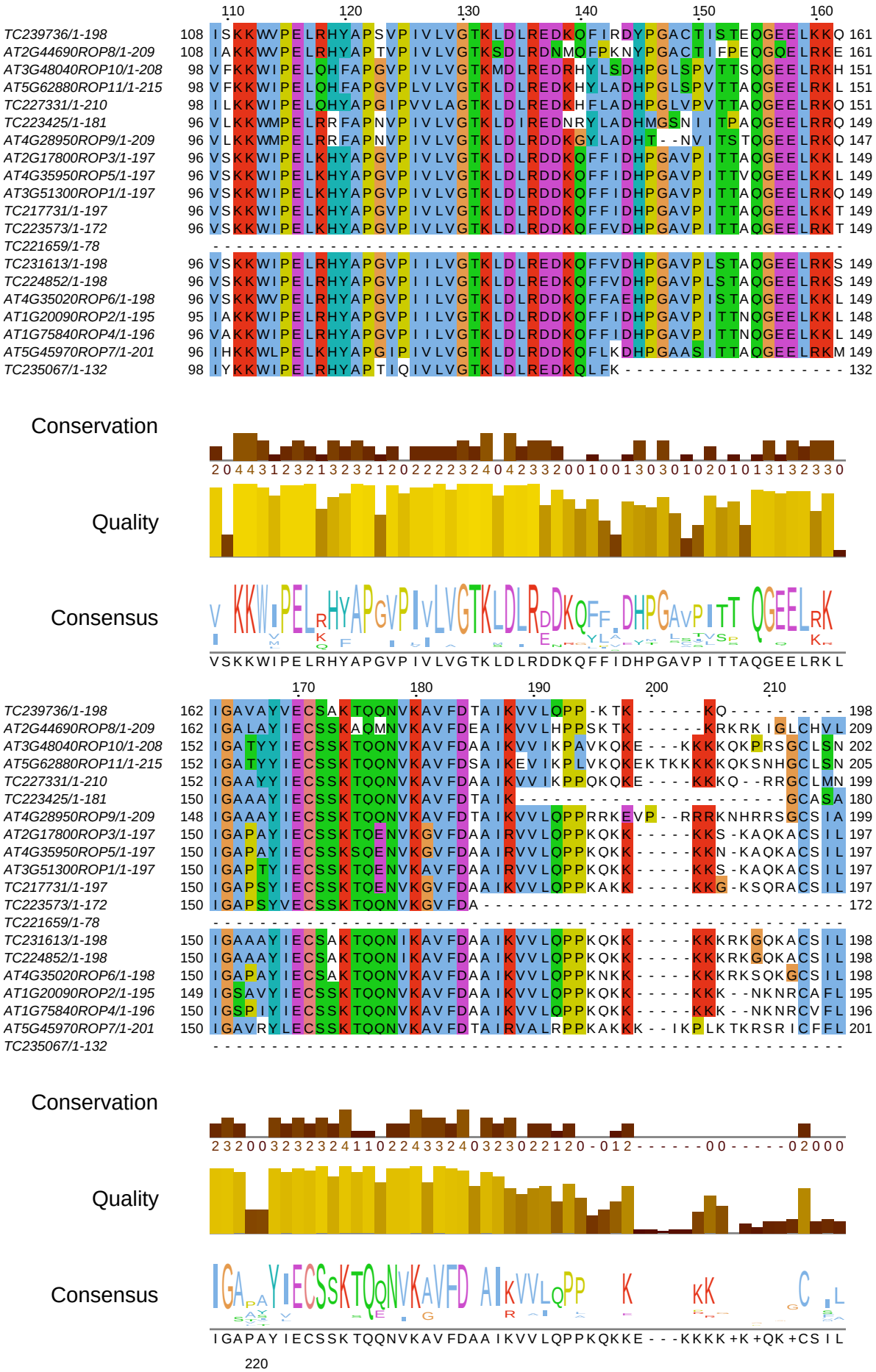


Fig 3.22 Phylogenetic tree of Arabidopsis and Tomato ROP GTPase The neighbour joining phylogenetic tree of Tomato and Arabidopsis ROP GTPase is shown with 1000 bootstrap values. TC239736 falls with AtROP8; TC 235067 falls with AtROP7, TC227331 falls with AtROP10 & 11; TC223425 falls along with AtROP9; TC231613 & TC224852 lie in the clade with AtROP6.



3.23 MSA of Arabidopsis & Tomato ROP GTPase.



3.23 MSA of Arabidopsis & Tomato ROP GTPase contt...

3.12.5 RAN GTPase

Nuclear RAS like GTPase (RAN) plays a crucial role in the nucleo-cytoplasmic transport, assembly of spindle fibres as well as it also pays an important role in the nuclear envelop organization. It is also important in the spatial and temporal organization of the cell. RAN GTPase is found in GDP bound form in cytoplasm during interphase and in an active GTP bound form during cell division. Plant RanGAP is targeted to the nuclear pore by a plant-specific mechanism involving two families of nuclear pore-associated proteins (Meier *et al*., 2010). Ran GTPase interacts with the RCC (regulator of chromosome condensation) motif thus regulating the microtubule dynamics. A total of 9 sequences for Ran GTPase were analysed from TIGR TC sequences and Ran like GTPase were found in tomato. Fig.3.24 shows the phyletic relationship and Fig.3.25 shows the MSA between the Arabidopsis and tomato Ran GTPase. The results indicate that there are at least 4 confirmed RAN GTPase. The TC237046, TC228522, TC2455029 which fall with the At RAN4 GTPase clade and rest with the TC219905. The other outliers falling in distant groups seems to be the errors of automated annotations and a result of incomplete and error prone ORFs. The future release of whole genome sequence will shed more light in the exact number of RAN GTPases in tomato. But for the current analysis 4 RAN GTPases in tomato can be confirmed with certainty.

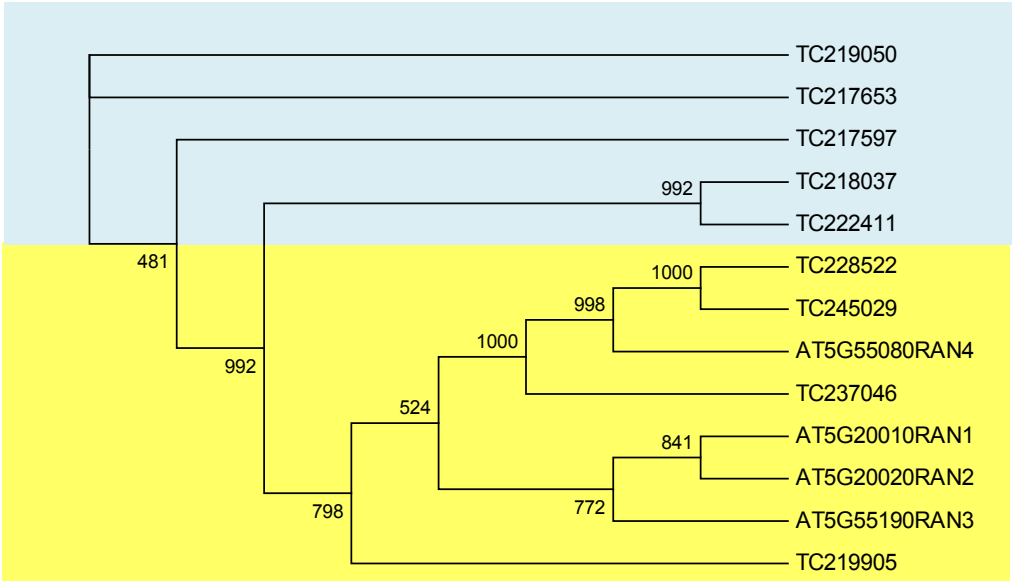
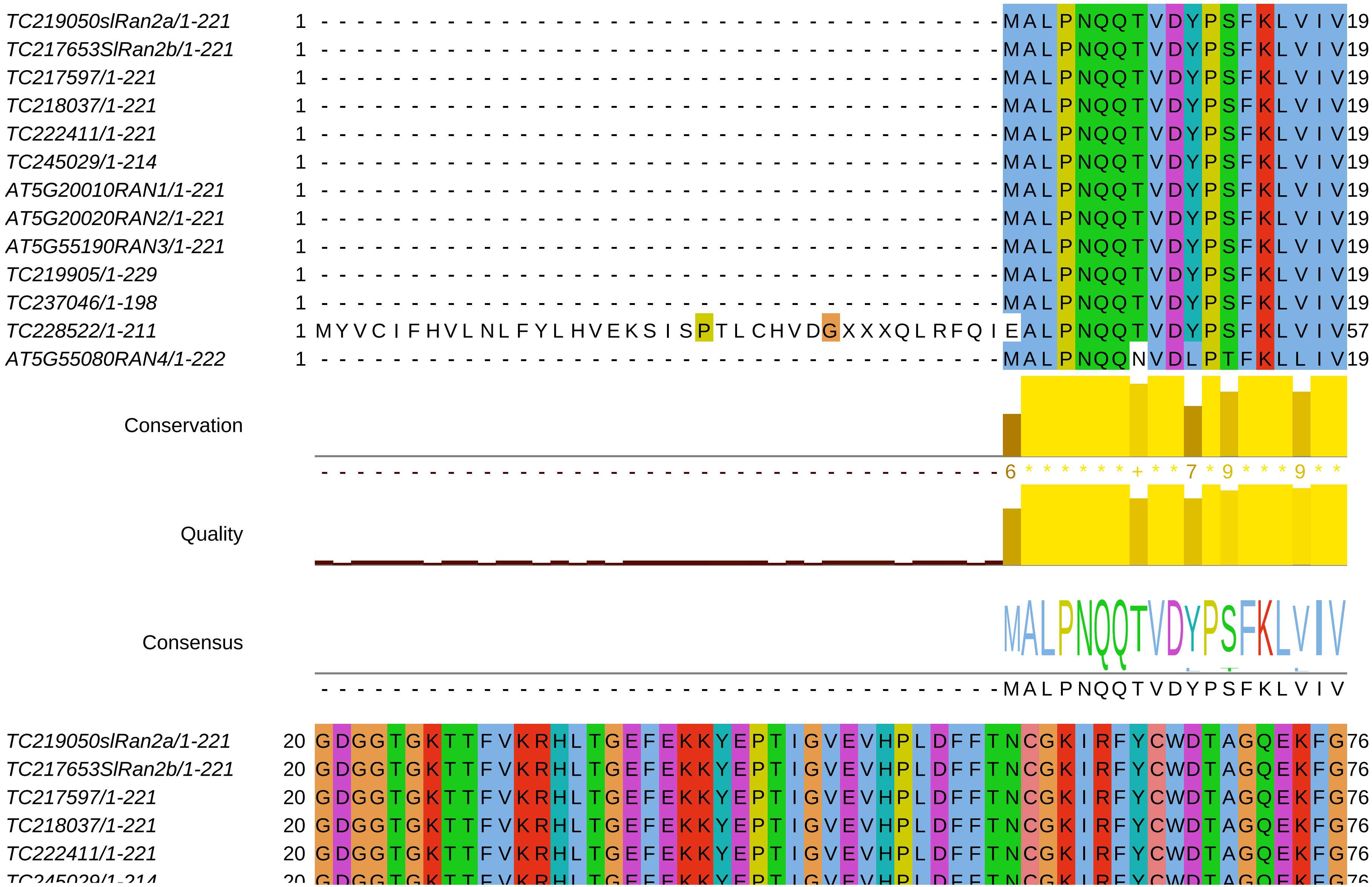
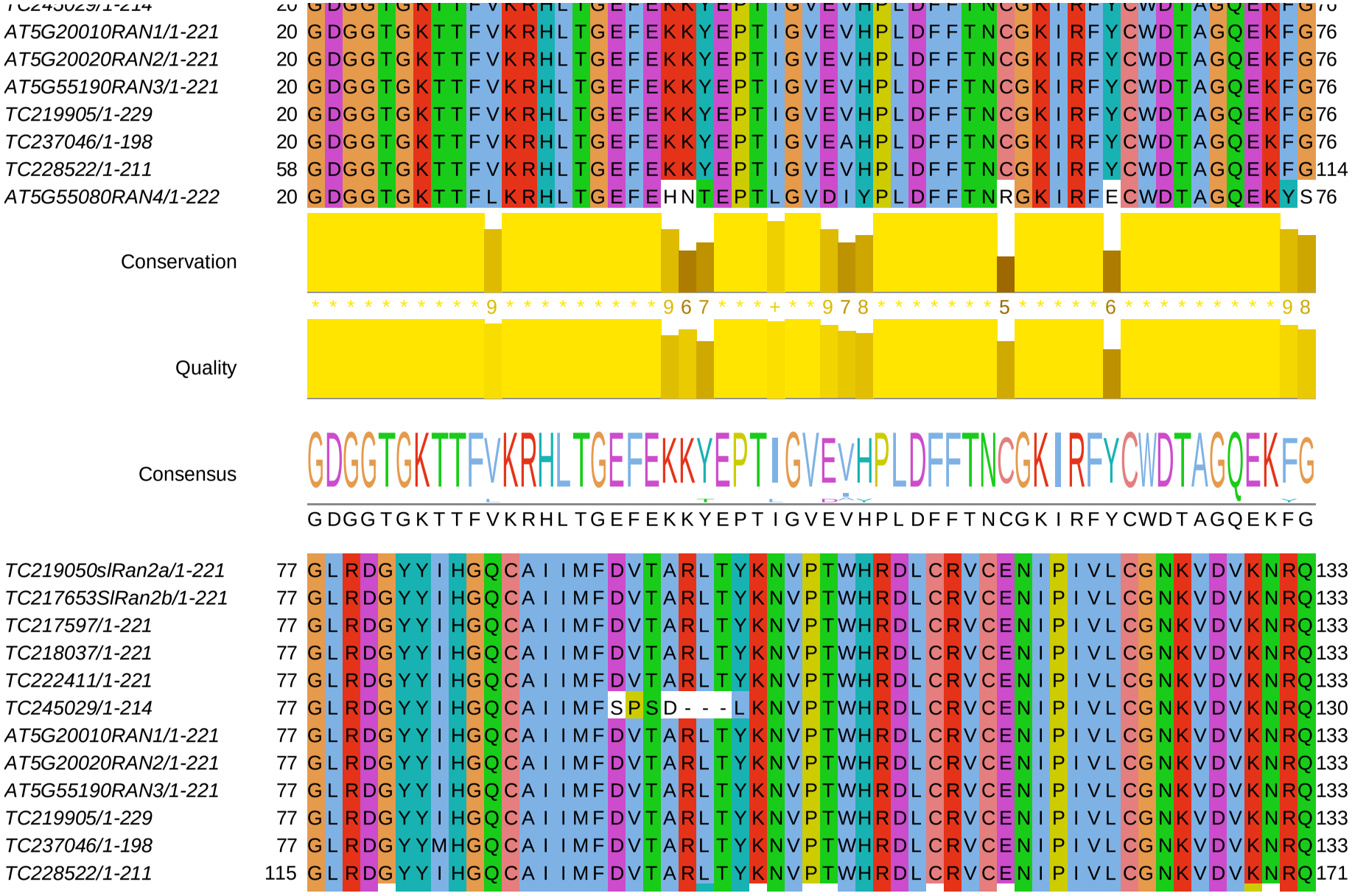


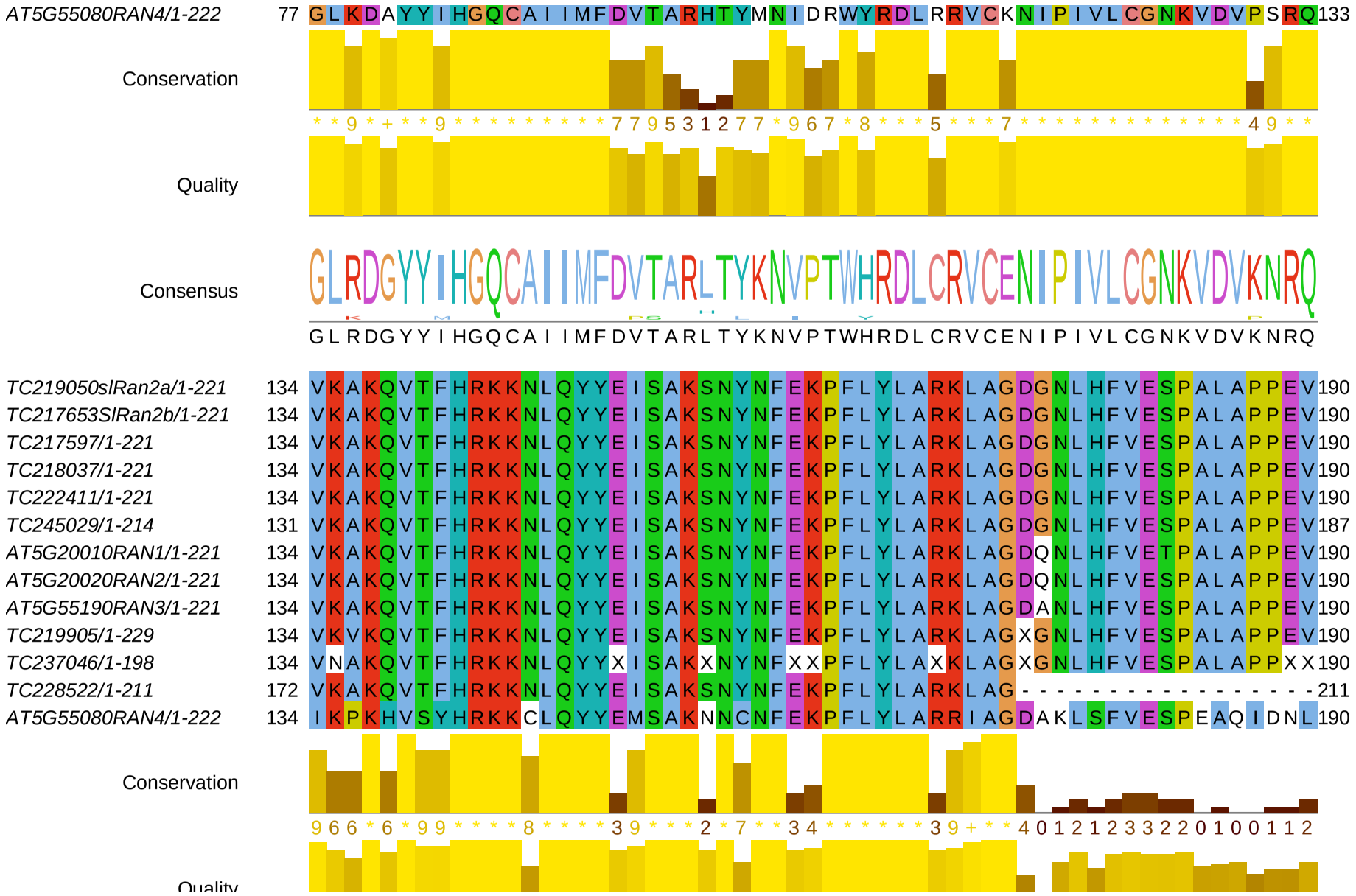
Fig 3.24 Phylogenetic tree of Arabidopsis and Tomato RAN GTPase: Neighbour Joining tree of At v/s Le Ran GTPase with 1000 boost strap values. The tree shows four confirmed RAN GTPase e.g. TC237046, TC228522, TC2455029 and TC219905. The other outliers could be RAN interacting partners or Ran GTP binding proteins (RanBP).



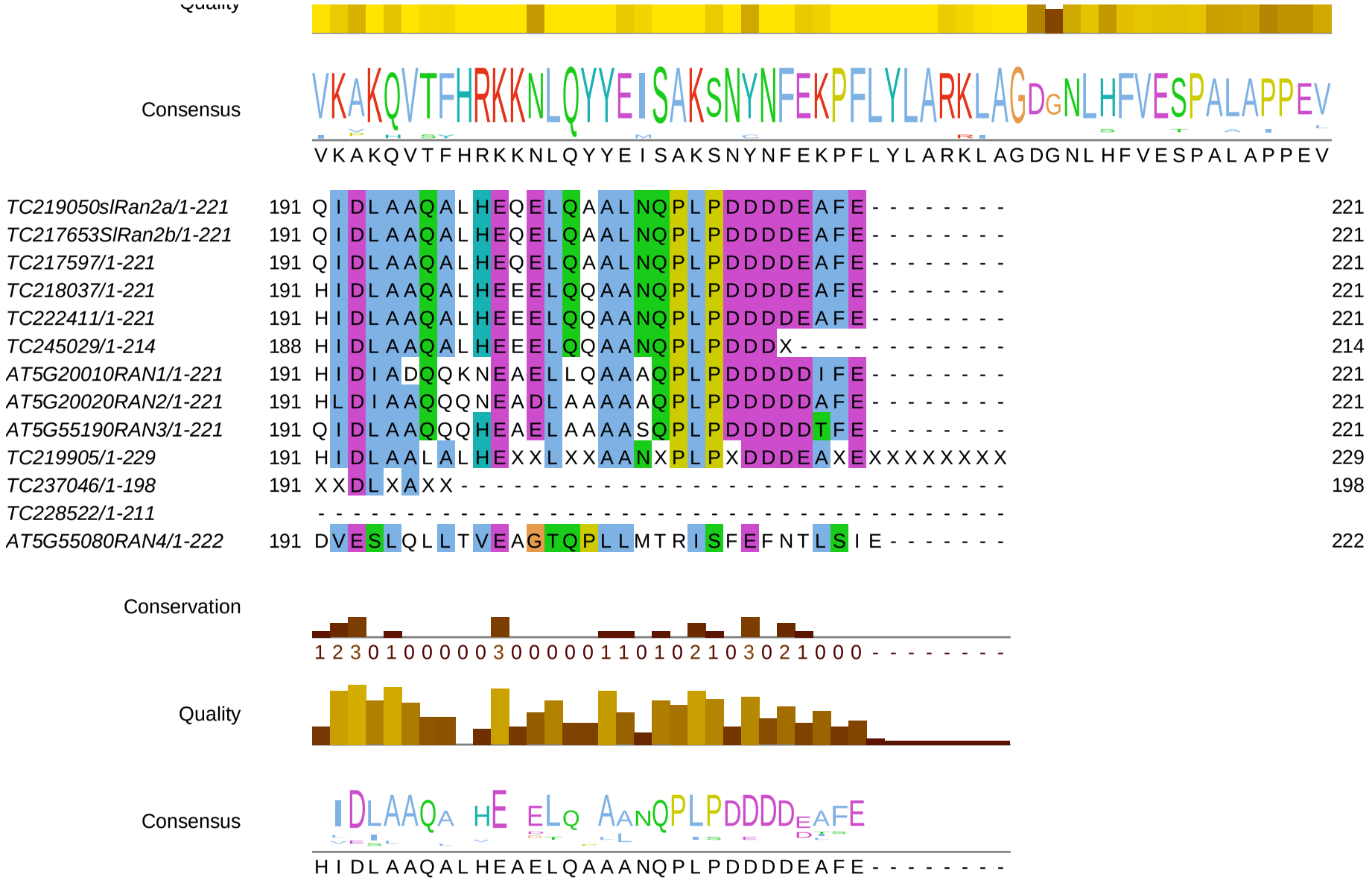
3.25 MSA of Arabidopsis & Tomato RAN GTPase.



3.25 MSA of Arabidopsis & Tomato RAN GTPase.



3.25 MSA of Arabidopsis & Tomato RAN GTPase cont...



3.25 MSA of Arabidopsis & Tomato RAN GTPase. The MSA was generated by JAlview2.8.2. The fig shows the Conservation, and group consensus logo of RAN GTPase.

3.12.6 Rab GAP

GTPase activator Protein (GAP) hydrolyses the GTP bound form of Rab to GDP bound form which is inactive state. Thus it helps in recycling the Rab and other GTPases. GAPs can be divided into several groups such as Rab GAP, Arf GAP, and RHO GAPs. There are 20 Rab GAPs in Arabidopsis, table 3.17 shows the Arabidopsis Rab GAPs obtained from TAIR database. These AGI codes were used to extract the sequence information for the blast search for tomato Rab GAP. In case of tomato a total of 23 Rab GAP were found and as shown in fig. 3.26 many of the tomato Rab GAP fall in the same cluster with the Arabidopsis Rab GAPs.

Table 3.17 Rab GAP in Arabidopsis

Rab GAP Group	Rab GAP	AGI Code
Rab GAPA	*AtGYPA1a*	At2g30710
	AtGYPA1b	At4g13730
	AtGYPA1c	At1g04830
Rab GAPB	*AtGYPB1a*	At2g20440
	AtGYPB1b	At4g28550
	AtGYPB1c	At4g27100
	AtGYPB1d	At5g54780
	AtGYPB1e	At2g43490
	AtGYPB1f	At3g57590
	AtGYPB2a	At5g52590
	AtGYPB2b	At5g41940
	AtGYPB2c	At5g53570
	AtGYPB2d	At3g49350
	AtGYPB2e	At5g24390
Rab GAPC	*AtGYPC1a*	At3g07890
	AtGYPC1b	At3g55020
	AtGYPC1c	At2g39280
	AtGYPC1d	At2g37290
Rab GAPD	*AtGYPD1a*	At3g02460
	AtGYPD1b	At5g15930

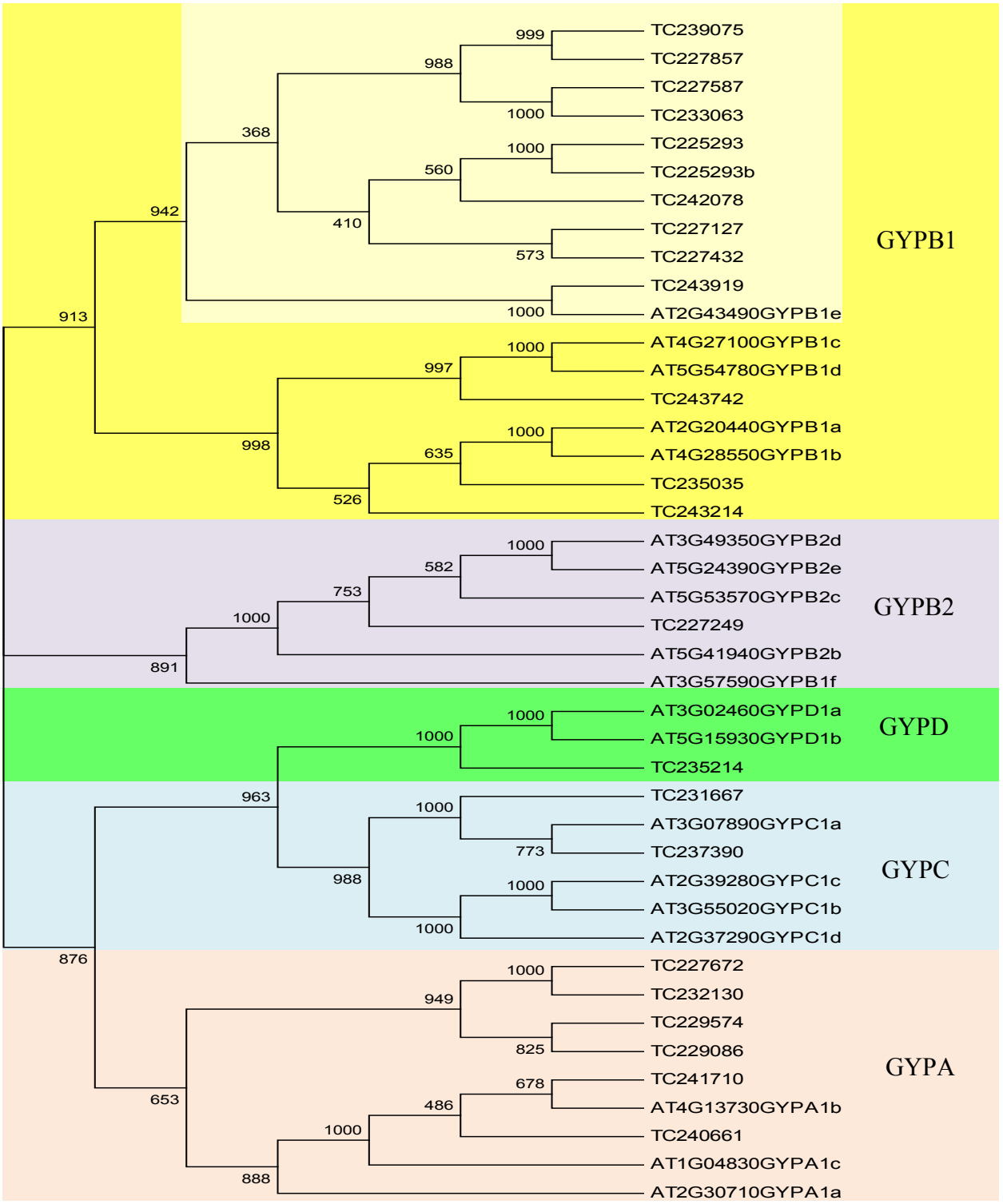


Fig 3.26 Phylogenetic tree of Arabidopsis and Tomato Rab GAP. Neighbour Joining tree of At v/s Le Rab GAP with 1000 boost strap values. Its is interesting to note that majority of the tomato RAB GAP fall within the clades of Arabidopsis Rab GAP but a more distant and distinct outlier group is also present which might be unique to tomato. A further detailed investigation and analysis is required with future release of tomato genomes sequence.

The nomenclature for Arabidopsis Rab GAP is given GYP because of the sequence similarity of YPT like GAP activity in case of yeast. There is one confirmed Rab GYP D (TC235214), two GYPC (TC231667, TC237390) and one GYPB2 (TC227249).

3.12.7 Rab GEF

GEF stands for Guanine nucleotide exchange factor. In case of Ras GTPases- the Ras GEF contains DP-PH tandem domain required for the docking of proteins. Rab GEFs are usually VPS9 and Sec2 proteins, ARF GEF have Sec7 domain whereas Ran GEF have a β-propeller protein containing RCC1 motif a regulator of chromosome condensation which is important in the transport of nuclear proteins. General mechanism of GEF's action includes often targeting the biological signal by inducing, inhibiting or modulating their catalytic activity.

GEF dissociates the nucleotides from the G-proteins by modifying the binding site, which results in the decreased affinity of nucleotide and thereby releasing it. The nucleotide of the G-protein is held in between the switch-I and switch-II region. This region interacts with the P-loop which is phosphate binding loop and it also coordinates with the magnesium ion, both PO^{4-} and Mg^{2+} are important for the nucleotide binding with G-proteins (Vetter and Wittinghofer, 2001).

The sequence information of the Arabidopsis Rab GEF was obtained from the TAIR database to use as reference to find the tomato Rab GEF from TIGR DFCI-TGI database. There are two Rab GEF in Arabidopsis AtVPS9a (At3g19770) and AtVPS9b (At5g09320). AtVPS9 is a Rab5 specific GEF (Goh *et al*., 2007; Nielsen et al., 2008). In case of the 12th release the Blast results indicated that there are at least 2 corresponding homologs of AtRabGEF in tomato *viz.* TC203309 (422aa), TC206589 (184aa). The sequence TC210626 (113aa) with incomplete ORF was not included in the study. Blast results in 13th release of TGI resulted in identification of two RabGEF in tomato *viz.* TC228769 and TC237702 (Release13). More concrete information will be available once the tomato genome sequence is out in future. GEF are important regulators of the RAB GTPase cycle cascade but still limited information is available on RabGEFs (Nielsen *et al*., 2008). It will be important to see if there are any class specific Rab GEFs, their mechanism of action as well as their expression at different stages may shed light on the Rab GTPase cycling mechanism. The fig. 3.27 shows the MSA of Arabidopsis and tomato Rab GEF with the conservation plots and consensus logo.

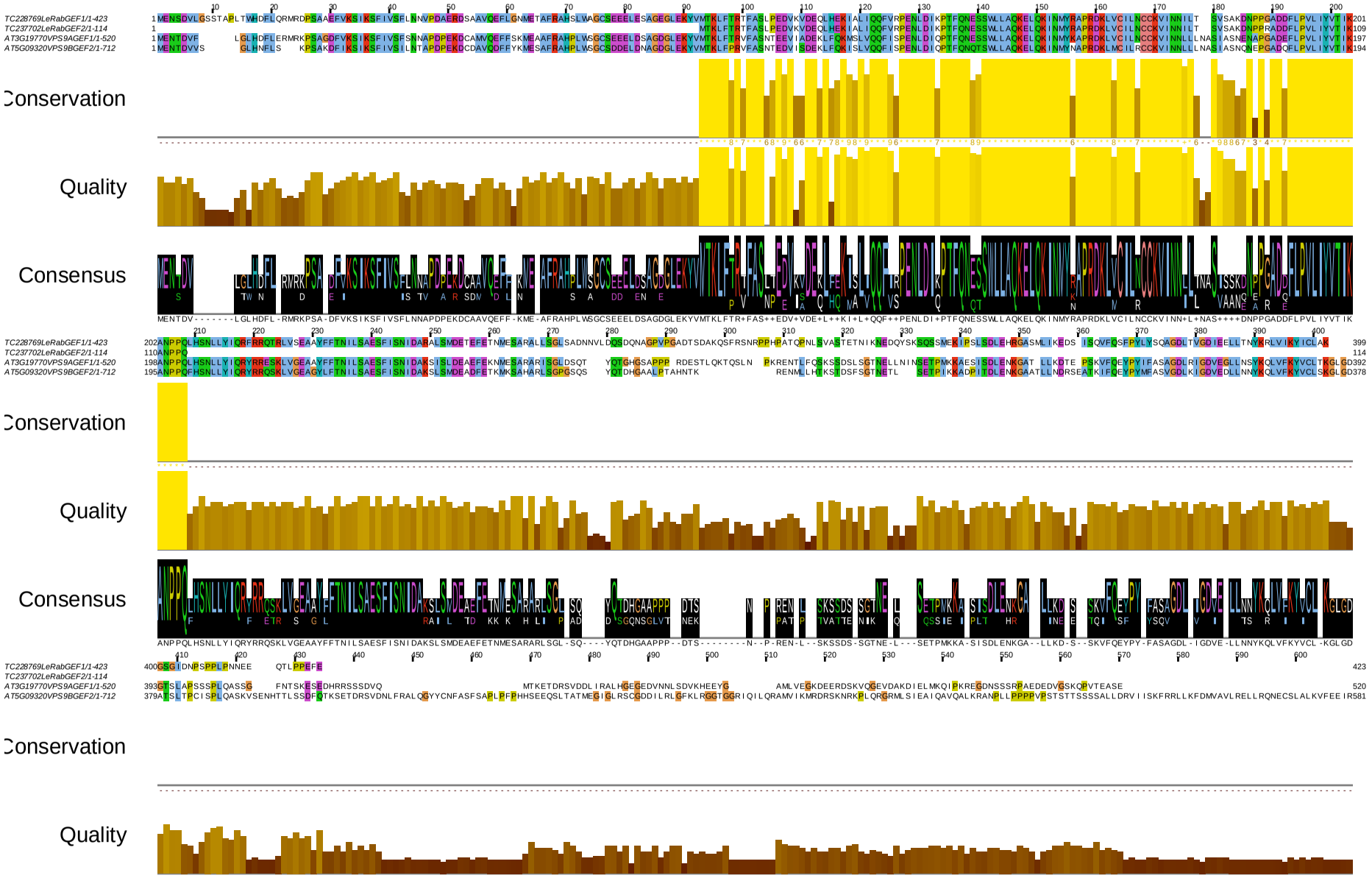


Fig3.27 Multiple Sequence alignment of Arabidopsis and Tomato RAB GEF.
Generated by Jalview 2.8.2 with Conservation plot, consensus histogram and logo of Rab GEF

3.12.8 Rab GDI

GDP dissociation inhibitor (GDI) interacts with the inactive state of Rab GDP and releases it into cytosol and play important role in translocation between the cytosol and cell membrane. The Rab Escort protein (REP) and Rab GDI are closely related in sequences and structure as well. Rab GDI play important role in the intracellular vesicle trafficking by escorting different GTPases between different endomembranes either from ER to Golgi or from Golgi to PM. Arabidopsis has 4 Rab GDI *viz*., AtRabGDI1(At3g59920); *AtRabGD1a(*At306540) *AtRabGDI2(*At2g44100) and *AtRabGDI3(*At5g09550). Six tomato Rab GDIs were found from the tomato gene index database according to the new 13th release. Fig. 3.28 shows the phylogenetic tree and Fig. 3.29 shows the MSA of Arabidopsis and Tomato RabGDI.

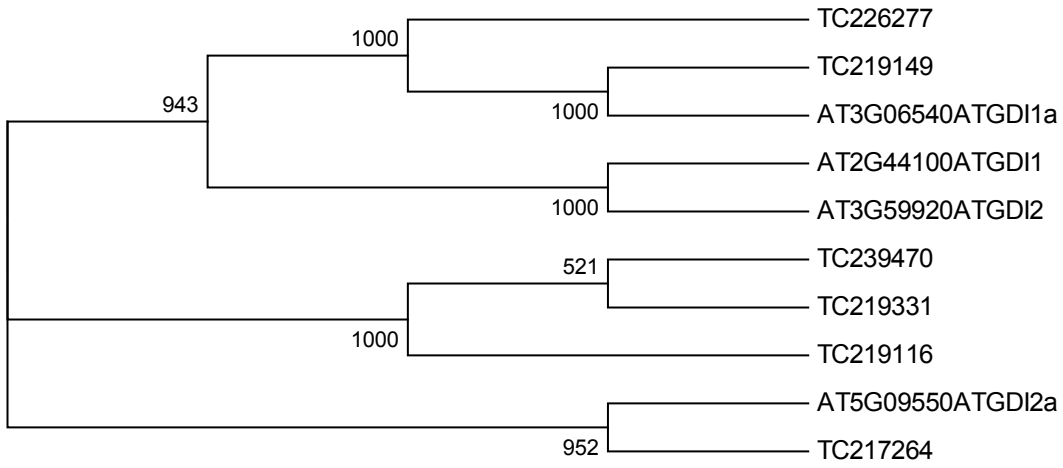


Fig 3.28 Phylogenetic tree of Arabidopsis and Tomato Rab GDI

Fig shows the neighbour joining tree of Arabidopsis and tomato Rab GDI with 1000 bootstrap value.

The phylogenetic analysis of Arabidopsis and tomato Rab GDI reveals that there is one confirmed GDI2 where in tomato TC217264 falls along with ATGDI2a. Likewise in case of AtGDI1 group two of the tomato TC226277 and TC219149 fall in one clade. There is one outlying group of sequences which forms a separate group altogether. It would be interesting to see once the final draft is out with correct annotation and detailed analysis as to which group these three sequences falls in case of tomato Rab GDIs.

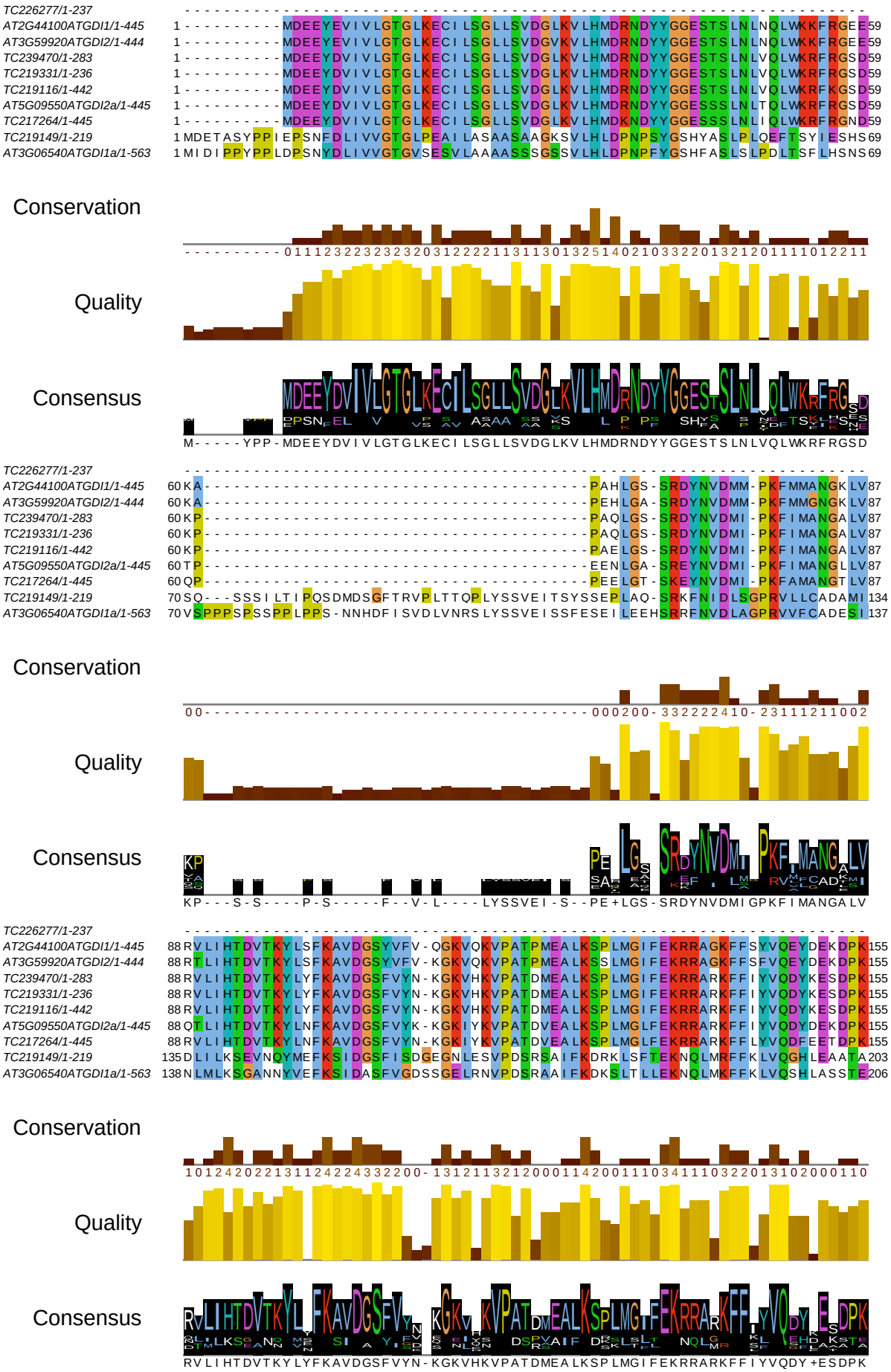


Fig. 3.29 **MSA of Arbidopsis and Tomato Rab GDI** generated by Jalview2.8.2 with conservation plot, histogram and consensus logo.

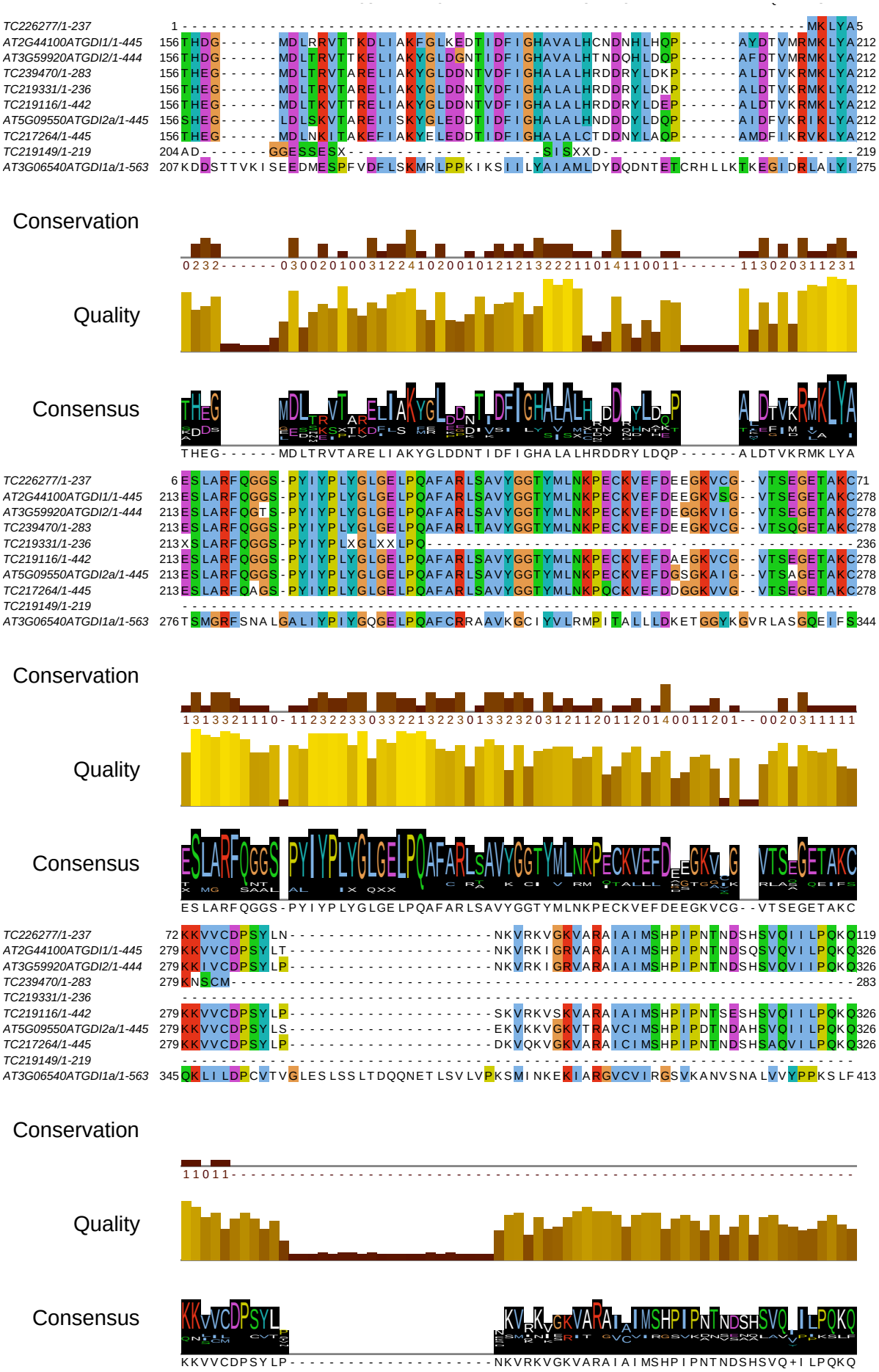


Fig. 3.29 MSA of Arbidopsis and Tomato Rab GDI cont...

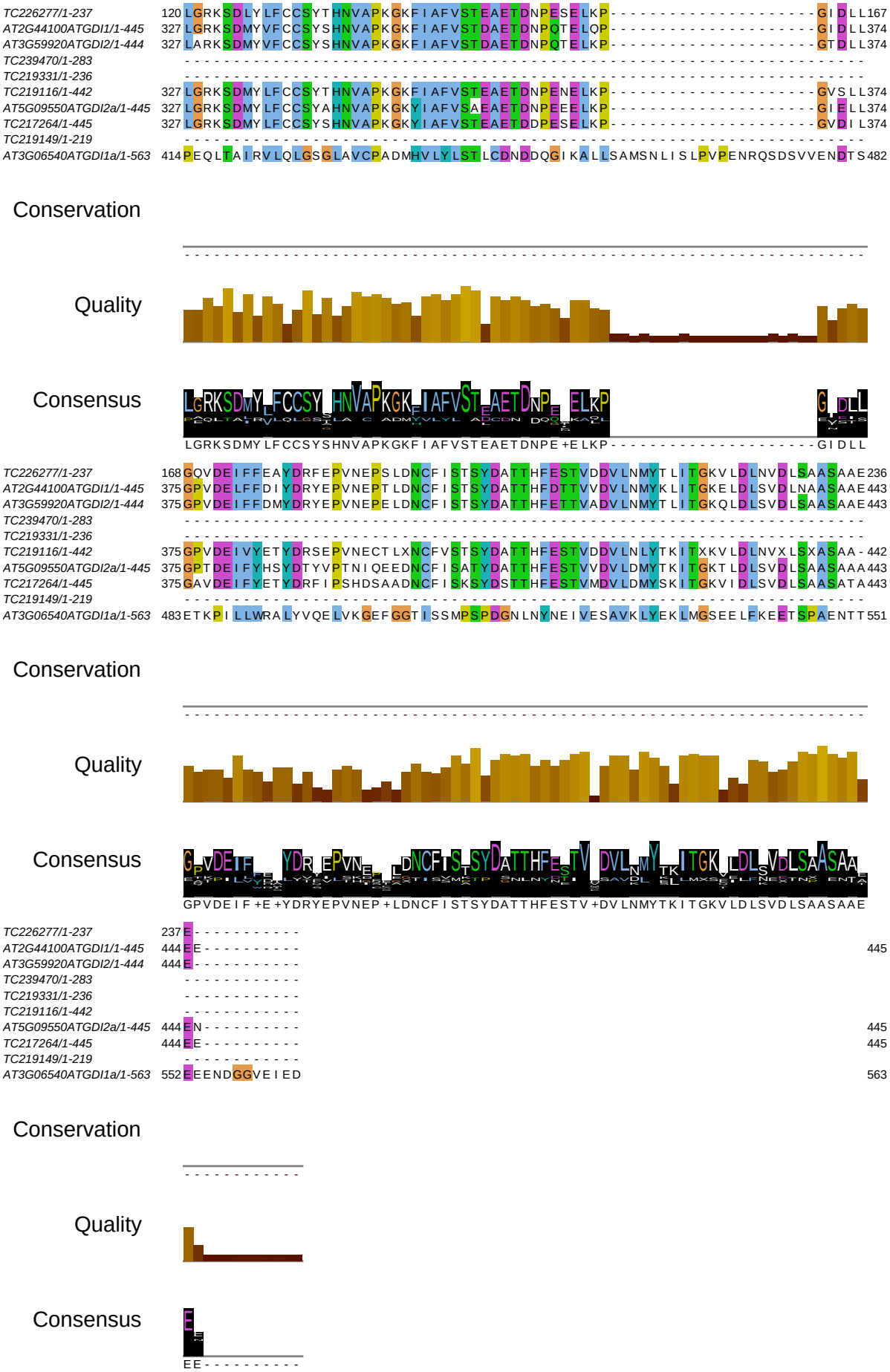


Fig. 3.29 MSA of Arbidopsis and Tomato Rab GDI cont...

3.13 *in silico* Analysis of Rab GTPase with Solyc IDs in the SGN Database with the current release of tomato whole genome sequence

Once the information for the Tomato Rab GTPases from (1) the TIGR Tomato gene index for TC Ids, (2) SGN database for Unigenes and (3) TED for Probe ids were retrieved and the identification, analysis, classification of tomato Rab GTPase was complete the next approach was to explore the recently released tomato whole genome sequence data at the SGN website. The same approach for the pathway and pipeline of analysis was applied for mining information for Rab GTPases in tomato genome database through the first to the current version of the ITAG release *i.e*. ITAG 1 to ITAG2.4 (Current release).

The protein sequences of Arabidopsis Rab GTPases were used as query to perform the BLAST search in SGN for Solyc IDs. Likewise the Rab GTPase identified from the TIGR database were also used as query for BLAST. The results of the BLAST outputs can be found in the supplemental files.

The preliminary analyses of whole genome database in SGN lead to the initial identification of different Rab GTPases with Solyc IDs in tomato. The information from the BLAST searches carried out by using the Arabidopsis and earlier identified Rab GTPase (TC ids) from the TIGR database as reference were also employed to hunt for Rab GTPases in tomato.

The list of putative Tomato Rab GTPase with Solyc Ids from SGN Database current version ITAG2.4 is shown in table 3.18. The initial results indicate that there are five Rab1/RabD, five Rab2/RabB, six Rab5/RabF, three Rab6/RabH, four Rab7/RabG, five Rab8/RabE GTPase, five Rab18/RabC and a total of 25 Rab11/RabA GTPases. These Solyc Ids show the automated annotations of Rab GTPase classes in the database. Although it's also important to check the sequences and the conserved domains and family specific motifs in order to validate the result and avoid the errors of false positive sequence annotation. Multiple sequence alignments of the individual class of Rab GTPase was analysed and the sequences were checked for the presence of group and class specific motifs and domains.

Table 3.18 List of Putative Tomato Rab GTPase Solyc Ids from SGN Database current version ITAG2.4

	Rab1/RabD		Rab2/RabB		Rab5/RabF
1	Solyc01g090170	1	Solyc02g093530	1	Solyc02g036450
2	Solyc01g103370	2	Solyc06g060210	2	Solyc02g069370
3	Solyc05g052070	3	Solyc07g056150	3	Solyc02g081380
4	Solyc05g053940	4	Solyc10g007700	4	Solyc05g045660
5	Solyc08g078070	5	Solyc12g011130	5	Solyc11g008430
				6	Solyc12g005840
	Rab6/RabH		**Rab7/RabG**		**Rab8/RabE**
1	Solyc01g088560	1	Solyc01g109520	1	Solyc04g011360
2	Solyc03g078570	2	Solyc03g120750	2	Solyc06g005350
3	Solyc05g051570	3	Solyc04g051680	3	Solyc09g010370
		4	Solyc04g072060	4	Solyc10g086350
				5	Solyc11g073050
	Rab11/A		**Rab18/RabC**		
1	Solyc01g086850	1	Solyc04g064510		
2	Solyc01g096220	2	Solyc07g052410		
3	Solyc01g103380	3	Solyc09g008460		
4	Solyc02g072180	4	Solyc10g086310		
5	Solyc03g006270	5	Solyc11g008020		
6	Solyc03g064020				
7	Solyc03g079900				
8	Solyc03g118820				
9	Solyc04g012180				
10	Solyc05g052900				
11	Solyc05g054150				
12	Solyc06g005810				
13	Solyc06g076450				
14	Solyc07g053480				
15	Solyc07g055290				
16	Solyc09g056340				
17	Solyc09g097900				
18	Solyc09g098170				
19	Solyc10g008840				
20	Solyc10g045550				
21	Solyc10g079030				
22	Solyc11g010100				
23	Solyc11g012460				
24	Solyc12g010790				
25	Solyc12g014080				

These are the putative Solyc IDs of Rab GTPase in tomato, and does not represent the final list. Sequences with incomplete ORFs will be excluded from further analysis and classification.

The annotations have remarkably improved with the release of the new annotation sets. Due to automated annotation and release of the newer version of ITAGs it was important to be careful as some times the annotations are misleading. The manual curation of the annotation is important. These are the initial screening results; more up to date information will be available in the future ITAG releases.

Table 3.19 shows the comparison of the Arabidopsis and Tomato Rab GTPase with the 'Solyc Ids' from Current SGN database ITAG2.4 release. The first column represents the class of Rab GTPase; the second column represents the sub family, followed by the AGI codes of Arabidopsis Rab GTPase and their corresponding Solyc Ids of tomato Rab GTPase with the amino acid length of the protein. The grouping represents only the tentative annotations with respect to release and more likely to change with future release and analysis.

Table 3.20 shows the functional annotation and locus position of Solyc Ids of tomato Rab GTPase in current SGN database. The information was mined from the database about the locus position, chromosome number on which the gene is present, its start and end position. The orientation of the gene is represented as + or – strand and the annotations by function is shown in the last column. For more details see the supplemental files in the (Folder name: Solyc BLAST results).

The report of Tomato locus LES245570 Rab11 GTPase is presented to give a brief idea about our target tomato Rab11a/RabA1a GTPase with the Solyc ID of Solyc07g055290 which is located on the chromosome 7 with id tag of SL2.50ch 07:63378793..63373504. The nucleotide sequence length of the gene is 5290 base pairs, the mRNA length is 1116 bp and the length of the protein is 219 AA. The gene structure of Rab11a is also highlighted in the report with corresponding SGN-Unigene Ids.

Tomato Rab11a GTPase (Solyc07g055290) was aligned with the Arabidopsis, Medicago and Soybean Rab GTPase (Fig.3.30) to show the conserved motifs and RAB specific domains with high homology in these crop species. Reference sequences tomato (Solyc07g055290); Arabidopsis (At1g06400), Medicago (Medtr2g083860); Soybean (Glyma13g36530, Glyma12g34000) were used.

Tomato Rab 11/RabA GTPase with Solyc Ids from SGN Database (ITAG2.4) are listed in the Table 3.21 with their corresponding locus ids and annotations, the SolycIDs with incomplete ORFs were not included in the analysis for the final list.

Table 3.19 Comparison of the Arabidopsis and Tomato Rab GTPase according to the Solyc Ids from Current SGN Database (AA-amino acid length)

Rab	Sub Group	Name	Arabidopsis AGI	AA	Tomato SolycIDs	AA
Rab A	**Rab A1**	Rab A1a	At1g06400	216	Solyc01g103380	214
		Rab A1b	At1g16920	216	Solyc03g079900	215
		Rab A1c	At5g45750	216	Solyc12g010790	217
					Solyc12g014080	217
		Rab A1d	At4g18800	214	Solyc10g045550	215
		Rab A1e	At4g18430	217	Solyc10g008840	215
		Rab A1f	At5g60860	217	Solyc07g053480	217
		Rab A1h	At2g33870	218	Solyc06g076450	215
		Rab A1g	At3g15060	217	Solyc07g055290	218
		Rab A1i	At1g28550	218	Solyc05g052900	215
	Rab A2	Rab A2b	At1g07410	214	Solyc02g072180	217
		Rab A2a	At1g09630	217	Solyc03g006270	216
		Rab A2c	At3g46830	217	Solyc06g005810	216
		Rab A2d	At5g59150	217		
	Rab A3	Rab A3	At1g01200	237	Solyc01g096220	243
	Rab A4	Rab A4a	At5g65270	226	Solyc04g012180	222
					Solyc11g010100	224
		Rab A4b	At4g39990	224		
		Rab A4c	At3g12160	222	Solyc01g086850	225
					Solyc03g064020	224
		Rab 4d	At5g47960	223	Solyc10g079030	226
		RabA4e	At2g22390	176		
	Rab A5	RabA5a	At5g47520	221	Solyc05g054150	226
					Solyc11g012460	276
		Rab A5b	At3g07410	217	Solyc09g097900	216
		Rab A5c	At2g43130	214	Solyc09g098170	217
		Rab A5d	At2g31680	219		
		Rab A5e	At1g05810	261		
	Rab A6	Rab A6a	At1g18200	229	Solyc03g118820	242
		Rab A6b	At1g73640	233		

Table 3.19 Comparison of the Arabidopsis and Tomato Rab GTPase according to the Solyc Ids from SGN Database (Continue…)

Rab	Sub Group	Name	Arabidopsis AGI	ORF	Tomato SolycID	ORF
Rab B	Rab B1	Rab B1a	At4g17160	205	Solyc06g060210	204
		Rab B1b	At4g17170	211	Solyc10g007700	211
					Solyc12g011130	211
		Rab B1c	At4g35860	211	Solyc02g093530	211
Rab C	RabC1	Rab C1	At1g43890	212	Solyc04g064510	209
	RabC2	Rab C2a	At5g03530	210	Solyc09g008460	216
					Solyc10g086310	209
		Rab C2b	At3g09910	205		
Rab D	RabD1	Rab D1	At3g11730	205	Solyc01g103370	202
	RabD2	Rab D2a	At1g02130	203	Solyc01g090170	203
		Rab D2b	At5g47200	202	Solyc05g053940	203
					Solyc08g078070	203
		Rab D2c	At4g17530	202	Solyc05g052070	203
RabE	Rab E1	Rab E1a	At3g53610	216	Solyc09g010370	216
		Rab E1b	At5g59840	216	Solyc11g073050	216
		Rab E1c	At3g46060	216	Solyc06g005350	144
		Rab E1d	At5g03520	216	Solyc10g086350	216
		Rab E1e	At3g09900	218	Solyc04g011360	216
RabF	RabF1	Rab F1	At3g54840	202	Solyc02g036450	200
	RabF2	Rab F2a	At5g45130	200	Solyc02g069370	200
		Rab F2b	At4g19640	200	Solyc02g081380	200
					Solyc11g008430	200
Rab G	Rab G1	Rab G1	At5g39620	204		
	Rab G2	Rab G2	At2g21880	212		
	RabG3	Rab G3a	At4g09720	217	Solyc03g120750	205
		Rab G3b	At1g22740	203	Solyc01g109520	206
		Rab G3c	At3g16100	206	Solyc04g072060	204
		Rab G3d	At1g52280	206	Solyc04g051680	219
		Rab G3e	At1g49300	206		
		Rab G3f	At3g18820	206		
Rab H	RabH1	Rab H1a	At5g64990	213	Solyc01g088560	208
		Rab H1b	At2g44610	208	Solyc03g078570	207
		Rab H1c	At4g39890	214	Solyc05g051570	207
		Rab H1d	At2g22290	207		
		Rab H1e	At5g10260	207		

Table 3.20 Functional annotation and locus position of Tomato Rab GTPase in current SGN database

Table 3.20.1 Functional Annotation of Solyc Rab1

mRNA ID	Solyc ID	Location	Start	End	Strand	Function
mRNA:Solyc01g090170.2.1	Solyc01g090170.2.1	Chromosome 01	75563002	75569993	+	Ras-related protein Rab-1A (AHRD V1 ***-C0HBJ4_SALSA);
mRNA:Solyc01g103370.2.1	Solyc01g103370.2.1	Chromosome 01	83750652	83755639	+	Ras-related protein Rab-1A (AHRD V1 ***-C0HBJ4_SALSA);
mRNA:Solyc05g052070.2.1	Solyc05g052070.2.1	Chromosome 05	61556718	61560014	-	Ras-related protein Rab-1A (AHRD V1 ***-B9EN43_SALSA)
mRNA:Solyc05g053940.2.1	Solyc05g053940.2.1	Chromosome 05	63104823	63109660	-	Ras-related protein Rab-1A (AHRD V1 ***-B6RB23_HALDI)
mRNA:Solyc08g078070.2.1	Solyc08g078070.2.1	Chromosome 08	59084544	59087746	+	Ras-related protein Rab-1A (AHRD V1 ***-B6RB23_HALDI)

Table 3.20.2 Functional annotation and locus position of Solyc Rab2

mRNA ID	Solyc ID	Location	Start	End	Strand	Function
mRNA:Solyc02g093530.2.1	Solyc02g093530.2.1	Chromosome02	48947510	48952106	-	Ras-related protein Rab-2-A (AHRD V1 B6T1U8_MAIZE)
mRNA:Solyc06g060210.1.1	Solyc06g060210.1.1	Chromosome06	34564883	34565494	+	Ras-related protein Rab-2-A (AHRD V1B6T1U8_MAIZE)
mRNA:Solyc07g056150.2.1	Solyc07g056150.2.1	Chromosome07	61318385	61322676	-	Ras-related protein Rab-2-A (AHRD V1 B6T1U8_MAIZE)
mRNA:Solyc10g007700.2.1	Solyc10g007700.2.1	Chromosome10	1953681	1958548	-	Ras-related protein Rab-2-A (AHRD V1 B6T1U8_MAIZE)
mRNA:Solyc12g011130.1.1	Solyc12g011130.1.1	Chromosome12	3971227	3974056	-	Ras-related protein Rab-2-A (AHRD V1B6T1U8_MAIZE)

Table 3.20.3 Functional annotation and locus position of Solyc Rab5

mRNA ID	Solyc ID	Location	Start	End	Strand	Function
mRNA:Solyc02g036450.2.1	Solyc02g036450.2.1	Chromosome02	21496239	21501862	-	Ras-related protein Rab-5C (AHRD V1 ***- C1BU54_9MAXI)
mRNA:Solyc02g069370.2.1	Solyc02g069370.2.1	Chromosome02	33859225	33867386	-	Ras-related protein Rab-5C (AHRD V1 ***- C1C0G6_9MAXI)
mRNA:Solyc02g081380.2.1	Solyc02g081380.2.1	Chromosome02	39946724	39951257	-	Ras-related protein Rab-5C (AHRD V1 ***- C1BU54_9MAXI)
mRNA:Solyc05g045660.2.1	Solyc05g045660.2.1	Chromosome05	56547429	56550136	+	Ras-related protein Rab-5C (AHRD V1 ***- B5X4R4_SALSA)
mRNA:Solyc11g008430.1.1	Solyc11g008430.1.1	Chromosome11	2614324	2620969	-	Ras-related protein Rab-5C (AHRD V1 ***- C1BU54_9MAXI)
mRNA:Solyc12g005840.1.1	Solyc12g005840.1.1	Chromosome12	474797	477406	-	Ras-related protein Rab-5C (AHRD V1 *-*- 5X4R4_SALSA)

Table 3.20.4 Functional annotation and locus position of Solyc Rab6

mRNA ID	Solyc ID	Location	Start	End	Strand	Function
mRNA:Solyc01g088560.2.1	Solyc01g088560.2.1	Chromosome01	75096820	75102455	-	Ras-related protein Rab-6A (AHRD V1 B6STE7_MAIZE)
mRNA:Solyc03g078570.2.1	Solyc03g078570.2.1	Chromosome03	44725181	44727743	-	Ras-related protein Rab-6A (AHRD V1 B6STE7_MAIZE)
mRNA:Solyc05g051570.2.1	Solyc05g051570.2.1	Chromosome05	61085144	61089191	-	Ras-related protein Rab-6A (AHRD V1 B6STE7_MAIZE)

Table 3.20.5 Functional annotation and locus position of Solyc Rab7

mRNA ID	Solyc ID	Location	Start	End	Strand	Function
mRNA:Solyc01g109520.2.1	Solyc01g109520.2.1	Chromosome01	88210021	88213641	-	Ras-related protein Rab-7 (AHRD V1 C1BJT6_OSMMO)
mRNA:Solyc03g120750.2.1	Solyc03g120750.2.1	Chromosome03	63080116	63084324	-	Ras-related protein Rab-7 (AHRD V1 C1BJT6_OSMMO)
mRNA:Solyc04g051680.2.1	Solyc04g051680.2.1	Chromosome04	50130142	50133500	-	Ras-related protein Rab-7 (AHRD V1 C1BJT6_OSMMO)
mRNA:Solyc04g072060.2.1	Solyc04g072060.2.1	Chromosome04	56710003	56720195	-	Ras-related protein Rab-7a (AHRD V1 C1C1X4_9MAXI)

Table 3.20.6 Functional annotation and locus position of Solyc Rab8

mRNA ID	Solyc ID	Location	Start	End	Strand	Function
mRNA:Solyc04g011360.2.1	Solyc04g011360.2.1	Chromosome04	3839267	3844110	-	Ras-related protein Rab-8A (AHRD V1 C1C362_9MAXI)
mRNA:Solyc06g005350.2.1	Solyc06g005350.2.1	Chromosome06	362156	370204	-	Ras-related protein Rab-8A (AHRD V1 C1BPQ4_9MAXI)
mRNA:Solyc09g010370.2.1	Solyc09g010370.2.1	Chromosome09	3753893	3759018	-	Ras-related protein Rab-8A (AHRD V1 C1C123_9MAXI)
mRNA:Solyc10g086350.1.1	Solyc10g086350.1.1	Chromosome10	64530098	64534665	-	Ras-related protein Rab-8A (AHRD V1 C1C362_9MAXI)
mRNA:Solyc11g073050.1.1	Solyc11g073050.1.1	Chromosome11	53248484	53252238	+	Ras-related protein Rab-8A (AHRD V1 C1C362_9MAXI)

Table 3.20.7 Functional annotation and locus position of Solyc Rab11

mRNA ID	Solyc ID	Location	Start	End	Strand	Function
mRNA:Solyc01g086850.2.1	Solyc01g086850.2.1	Chromosome01	73531859	73536531	-	Ras-related protein Rab-25 (AHRD V1 ***-RAB25_HUMAN)
mRNA:Solyc01g096220.2.1	Solyc01g096220.2.1	Chromosome01	79075516	79077682	+	Ras-related protein Rab-25 (AHRD V1 ***-RAB25_BOVIN)
mRNA:Solyc01g103380.2.1	Solyc01g103380.2.1	Chromosome01	83755957	83758949	-	Ras-related protein Rab-25 (AHRD V1 ***-RAB25_MOUSE)
mRNA:Solyc02g072180.2.1	Solyc02g072180.2.1	Chromosome02	36074672	36080983	+	Ras-related protein Rab-25 (AHRD V1 ***-RAB25_BOVIN)
mRNA:Solyc03g006270.2.1	Solyc03g006270.2.1	Chromosome03	888904	890977	+	Ras-related protein Rab-25 (AHRD V1 ***-RAB25_BOVIN)
mRNA:Solyc03g064020.2.1	Solyc03g064020.2.1	Chromosome03	34600518	34601681	+	Ras-related protein Rab-25 (AHRD V1 ***-RAB25_HUMAN)
mRNA:Solyc03g079900.2.1	Solyc03g079900.2.1	Chromosome03	45334174	45335900	+	Ras-related protein Rab-25 (AHRD V1 ***-RAB25_MOUSE)
mRNA:Solyc03g118820.2.1	Solyc03g118820.2.1	Chromosome03	61679372	61682468	+	Ras-related protein Rab-25 (AHRD V1 ***-RAB25_BOVIN)
mRNA:Solyc04g012180.2.1	Solyc04g012180.2.1	Chromosome04	4461532	4467418	-	Ras-related protein Rab-25 (AHRD V1 ***-RAB25_RABIT)
mRNA:Solyc05g052900.2.1	Solyc05g052900.2.1	Chromosome05	62223052	62225281	+	Ras-related protein Rab-25 (AHRD V1 ***-RAB25_BOVIN)
mRNA:Solyc05g054150.2.1	Solyc05g054150.2.1	Chromosome05	63253042	63255141	+	Ras-related protein Rab-25 (AHRD V1 ***-RAB25_MOUSE)
mRNA:Solyc06g005810.2.1	Solyc06g005810.2.1	Chromosome06	839934	842843	-	Ras-related protein Rab-25 (AHRD V1 ***-RAB25_MOUSE)
mRNA:Solyc06g076450.2.1	Solyc06g076450.2.1	Chromosome06	43888198	43890284	-	Ras-related protein Rab-25 (AHRD V1 ***-RAB25_MOUSE)
mRNA:Solyc07g053480.2.1	Solyc07g053480.2.1	Chromosome07	59251334	59254015	+	Ras-related protein Rab-25 (AHRD V1 ***-RAB25_BOVIN)
mRNA:Solyc07g055290.2.1	Solyc07g055290.2.1	Chromosome07	60697004	60702293	-	Ras-related protein Rab-25 (AHRD V1 ***-RAB25_BOVIN)
mRNA:Solyc09g056340.2.1	Solyc09g056340.2.1	Chromosome09	44378803	44381132	+	Ras-related protein Rab-25 (AHRD V1 ***-RAB25_RABIT)
mRNA:Solyc09g097900.2.1	Solyc09g097900.2.1	Chromosome09	67163971	67169072	-	Ras-related protein Rab-25 (AHRD V1 ***-RAB25_RABIT)
mRNA:Solyc09g098170.2.1	Solyc09g098170.2.1	Chromosome09	67317558	67321196	+	Ras-related protein Rab-25 (AHRD V1 ***-RAB25_RABIT)
mRNA:Solyc10g008840.2.1	Solyc10g008840.2.1	Chromosome10	2900438	2901941	+	Ras-related protein Rab-25 (AHRD V1 ***-RAB25_BOVIN)..

mRNA:Solyc10g045550.1.1	Solyc10g045550.1.1	Chromosome10	30059252	30065074	+	Ras-related protein Rab-25 (AHRD V1 ***-RAB25_BOVIN)
mRNA:Solyc10g079030.1.1	Solyc10g079030.1.1	Chromosome10	59995245	59996726	-	Ras-related protein Rab-25 (AHRD V1 ***-RAB25_RABIT)
mRNA:Solyc11g010100.1.1	Solyc11g010100.1.1	Chromosome11	3222459	3224358	+	Ras-related protein Rab-25 (AHRD V1 ***-RAB25_BOVIN)
mRNA:Solyc11g012460.1.1	Solyc11g012460.1.1	Chromosome11	5297608	5298810	+	Ras-related protein Rab-25 (AHRD V1 ***-RAB25_RABIT)
mRNA:Solyc12g010790.1.1	Solyc12g010790.1.1	Chromosome12	3711266	3713629	-	Ras-related protein Rab-25 (AHRD V1 ***-RAB25_BOVIN)
mRNA:Solyc12g014080.1.1	Solyc12g014080.1.1	Chromosome12	4894053	4896737	-	Ras-related protein Rab-25 (AHRD V1 ***-RAB25_BOVIN)

Table 3.20.8 Functional annotation and locus position of Solyc Rab18

mRNA ID	Solyc ID	Location	Start	End	Strand	Function
mRNA:Solyc04g064510.2.1	Solyc04g064510.2.1	Chromosome04	54789382	54794367	-	Ras-related protein Rab-18 (AHRD V1 ***-B6TSE5_MAIZE)
mRNA:Solyc07g052410.2.1	Solyc07g052410.2.1	Chromosome07	58250571	58252629	+	Ras-related protein Rab-18 (AHRD V1 ***-B6TGJ7_MAIZE)
mRNA:Solyc09g008460.2.1	Solyc09g008460.2.1	Chromosome09	1905656	1911197	-	Ras-related protein Rab-18 (AHRD V1 ***-B6TSE5_MAIZE)
mRNA:Solyc10g086310.1.1	Solyc10g086310.1.1	Chromosome10	64502213	64503613	-	Ras-related protein Rab-18 (AHRD V1 ***-B6TSE5_MAIZE)
mRNA:Solyc11g008020.1.1	Solyc11g008020.1.1	Chromosome11	2238213	2241626	+	Ras-related protein Rab-18 (AHRD V1 ***-B6TSE5_MAIZE)

The above tables of Rab GTPase with SolycIDs were mined from the SGN database based on the respective annotations as shown in the last column. The sequences were visually inspected for the complete ORFs. Those sequences with incomplete ORFs were excluded from the further analysis, including half length protein sequences or the sequences with the additional large chunks not characteristic of the Rab family which may lead to erroneous alignment and may result in false positive grouping in the phylogenetic trees. These results are discussed further in the discussion chapter.

Tomato locus LES245570 Rab11 GTPase Report

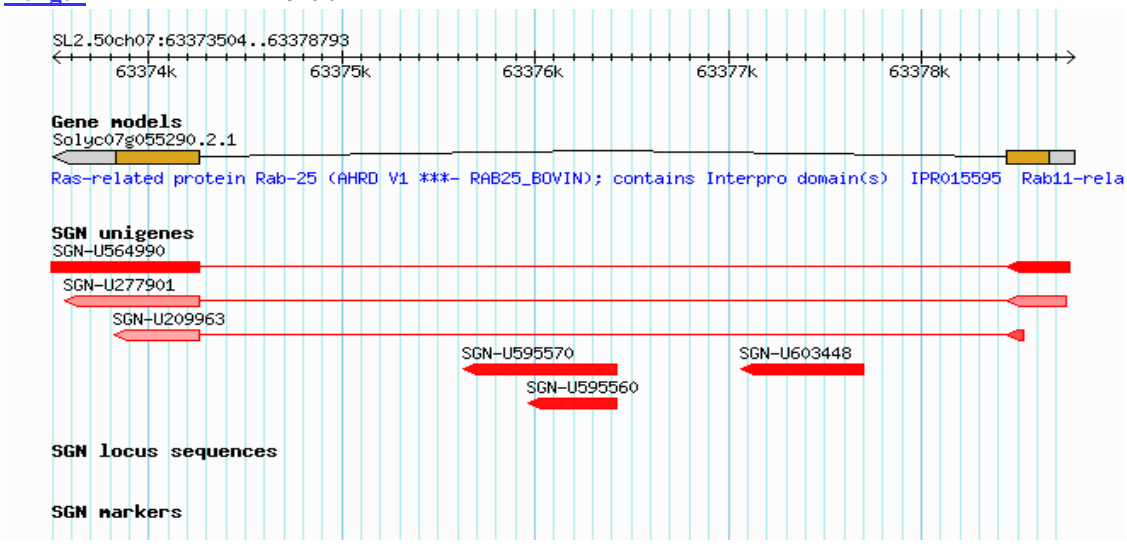
Locus **Solyc07g055290**
Locus name **LES245570 Rab11 GTPase**
Symbol ***rab11a***
Chromosome **7** Arm
Locus synonyms 2: Rab11a Solyc07g055290
Created on: 2006-05-10 Last updated on: 2010-11-24 by Naama Menda
Annotation Set ITAG2.4 Release: genomic annotations
Sequence Region Solyc07g055290.2.1:1..219
Feature(s) matched Solyc07g055290.2.1
SGN Unigenes SGN-U564990 Tomato 200607 -- build 2 -- 18 members
GenBank accessions AJ245570 Lycopersicon esculentum mRNA for Rab11 GTPase (Rab11a gene).
PMID:11487695 Developmental abnormalities and reduced fruit softening in tomato plants expressing an antisense Rab11 GTPase gene. (2001)
Ontology Annotation

biological_process
involved in intracellular protein transport (GO: 0006886)
involved in small GTPase mediated signal transduction (GO: 0007264)
involved in protein transport (GO: 0015031)

cellular_component
located in exosome (RNase complex) (GO: 0000178)
located in intracellular (GO: 0005622)

Solanum lycopersicum gene Solyc07g055290.2

Name	Solyc07g055290.2
Type	Gene
Organism	*Solanum lycopersicum*
Synonyms / aliases	Solyc07g055290
Length	5,290
Description	Ras-related protein Rab-25 (AHRD V1 ***- RAB25_BOVIN); contains Interpro domain(s) IPR015595 Rab11-related
Location(s)	SL2.50ch07:63378793...63373504
GFF source	ITAG_eugene
From BOGAS	1
Length	5290



The above image shows the gene structure of the tomato Rab11a/RabA1a GTPase and the SGN Unigene id.

Annotation Set
ITAG2.4 Release: genomic annotations

Sequence Region
SL2.50ch07:63373504..63378793

Type	Name	Location	Length	Strand
mRNA	Solyc07g055290.2.1	SL2.50ch07:63373504..63378793	1,116	-

```
>Solyc07g055290.2.1 Ras-related protein Rab-25 (AHRD V1 ***- RAB25_BOVIN);
contains Interpro domain(s) IPR015595 Rab11-related
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Amino Acid Sequence

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MAGYRGDDEYDYLFKLVLIGDSGVGKSNLLSRFTKNEFNLESKSTIGVEFATKSLNIDGKVIKAQIWDTAGQER
YRAITSAYYRGAVGALLVYDVTRHVTFENVNRWLKELRDHTDPNIVVMLLGNKSDLRHLVAVSTEEAKSLAERE
ALYFMETSALEATNVENAFTEALTQIYRIVSKKAVEAGDEGATSTAPPKGQTINIKDEGSAWKKFGCCSS*
```

Type	Name	Location	Length	Strand
polypeptide	Solyc07g055290.2.1	SL2.50ch07:63373828..63378659	219	-

Add accessions to a list:

Features this mRNA is a part of

Type	Name	Location	Length	Strand	Phase
gene	Solyc07g055290.2	SL2.50ch07:63373504..63378793	5,290	-	n/a

Features that are parts of this mRNA

Type	Name	Location	Length	Strand
exon	exon:Solyc07g055290.2.1.1	SL2.50ch07:63378439..63378793	355	-
exon	exon:Solyc07g055290.2.1.2	SL2.50ch07:63373504..63374264	761	-
intron	intron:Solyc07g055290.2.1.2	SL2.50ch07:63374265..63378438	n/a	-

Genetic loci Tomato locus rab11a (LES245570 Rab11 GTPase)

Annotation Set	Feature(s)	Region
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ITAG2.4 Release: genomic annotations	Solyc07g055290.2	SL2.50ch07:63373504..63378793
ITAG2.4 Release: genomic annotations	Solyc07g055290.2.1	Solyc07g055290.2.1
ITAG2.3 Release: protein annotations	Solyc07g055290.2.1	Solyc07g055290.2.1
ITAG2.3 Release: genomic annotations	Solyc07g055290.2	SL2.40ch07:60697004..60702293
ITAG2 Release: genomic annotations	Solyc07g055290	SL2.31ch07:60700203..60705033

ITAG2.4 Release: genomic annotations: 5.38 kbp from SL2.50ch07:63,373,387...63,378,766

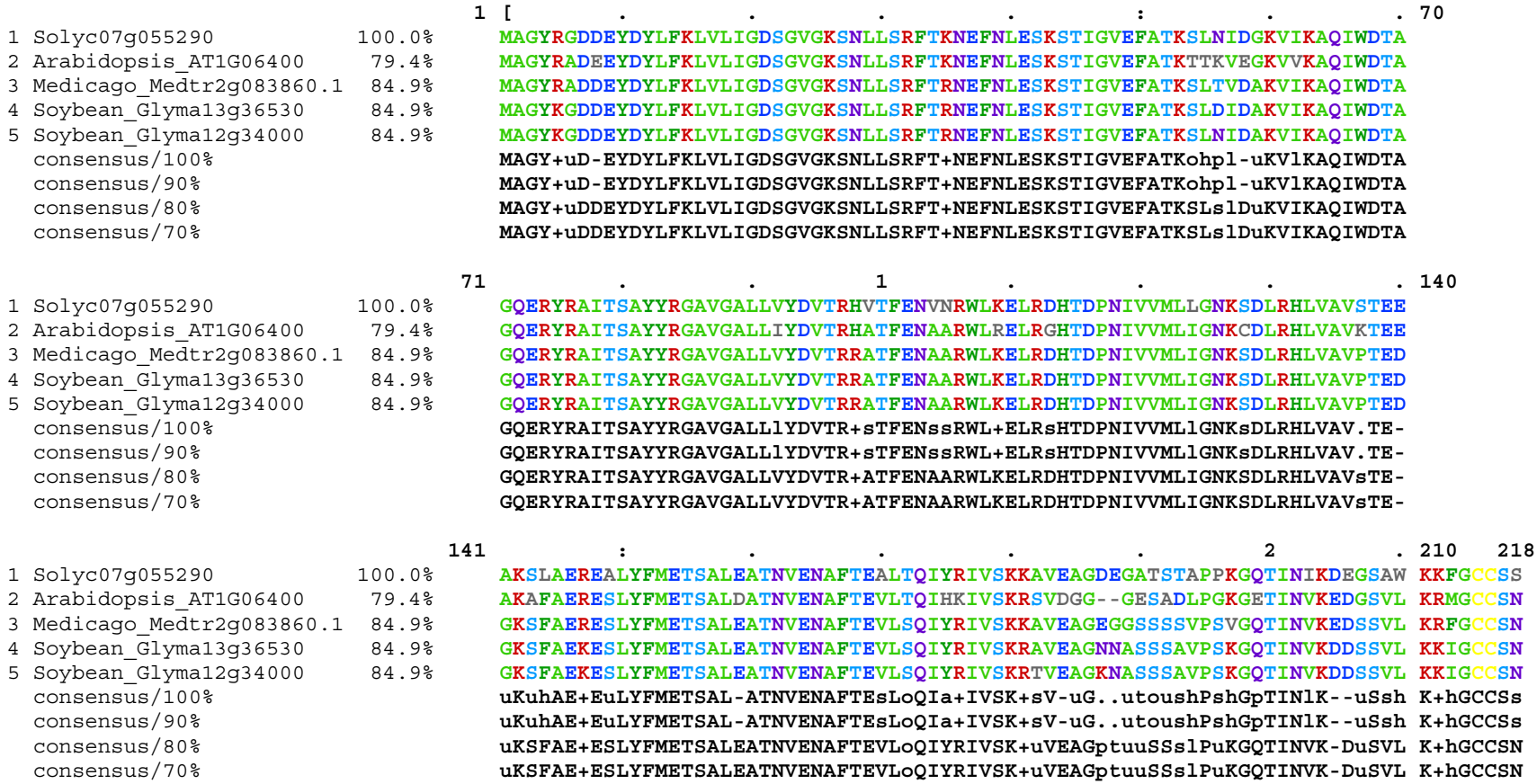


Fig.3.30 MSA of At, Solyc, Medtr and Glyma RabA1a/Rab11a sequence

Tomato Rab11 GTPase (Solyc07g055290) was aligned with the Arabidopsis, Medicago and Soybean Rab GTPase to show the conserved motifs and RAB specific domains with high homology in different crop species. Reference sequences tomato (Solyc07g055290); Arabidopsis (At1g06400), Medicago (Medtr2g083860); Soybean(Glyma13g36530, Glyma12g34000). Identities normalised by aligned length. The above MSA is generated by using the BioMview (MView 1.51) software.

Table 3.21 Annotation of Tomato Rab11/ RabA Solyc Ids from SGN (ITAG2.4)

Sr	SolycID	Rab	Locus name	Chr		NT	mRNA	AA	Ex	Annotation
1	Solyc01g086850	Rab11	Solyc01g086850	1	-	4,673	1,194	226	2	Rab11-related AtRABA4c
2	Solyc01g096220	Rab11	Solyc01g096220	1	+	2167	1,170	244	3	Rab11-related AtRABA3 Rab11D
3	Solyc01g103380	Rab11	Solyc01g103380	1	-	2993	1,029	215	2	Rab11-related ATRABA2A, ATRAB11C
4	Solyc02g072180	Rab11	Solyc02g072180	2	+	6,312	1,082	218	2	Rab11-related AtRABA1d/AtRab11B
5	Solyc03g006270	Rab11	Solyc03g006270	3	+	2,074	1,136	217	2	Rab11-related AtRABA1f
6	Solyc03g064020	Rab11	Solyc03g064020	3	+	1,164	883	225	2	Rab11-related
7	Solyc03g079900	Rab11	Solyc03g079900	3	+	1,727	703	216	3	Rab11-related
8	Solyc03g118820	Rab11	Solyc03g118820	3	+	3,097	797	243	4	Rab11-related AtRABA6a
9	Solyc04g012180	Rab11	Solyc04g012180	4	-	5,887	1,067	223	2	Rab11-related Rab11D AtRABA4a
10	Solyc05g052900	Rab11	Solyc05g052900	5	+	2,230	737	216	2	Rab11-related
11	Solyc05g054150	Rab11	Solyc05g054150	5	+	2,100	1,272	227	2	Rab11-related AtRABA5a
12	Solyc06g005810	Rab11	Solyc06g005810	6	-	2,910	1,046	217	3	Rab11-related AtRABA2b AtRABA2c
13	Solyc06g076450	Rab11	Solyc06g076450	6	-	2087	1,146	216	3	Rab11-related AtRABA2b
14	Solyc07g053480	Rab11	Solyc07g053480	7	+	2682	1,076	218	2	Rab11-related AtRABA1f Rab11B
15	Solyc07g055290	Rab11	LES245570 Rab11	7	-	5290	1,116	219	2	***rab11a*** AtRABA1a,
16	Solyc09g056340	Rab11	Solyc09g056340	9	+	2,330	1,070	211	2	Rab11-related AtRABA5b
17	Solyc09g097900	Rab11	Solyc09g097900	9	-	5,102	975	217	2	Rab11-related AtRABA5d
18	Solyc09g098170	Rab11	Solyc09g098170	9	+	3,639	1,040	218	2	Rab11-related AtRab11D, AtRABA5e
19	Solyc10g008840	Rab11	Solyc10g008840	10	+	1,504	1,019	216	2	Rab11-related AtRABA1f Rab11B
20	Solyc10g045550	Rab11	Solyc10g045550	10	+	5,823	648	216	2	Rab11-related ATRABA2A, ATRAB11C
21	Solyc10g050030	Rab11	Solyc10g050030	10	+	1,360	240	80	3	Rab11-related AA Length 80
22	Solyc10g079030	Rab11	Solyc10g079030	10	-	1,482	681	227	2	Rab11-related
23	Solyc11g010100	Rab11	Solyc11g010100	11	+	1,900	675	225	2	Rab11-related AtRABA4a
24	Solyc11g012460	Rab11	Solyc11g012460	11	+	1,203	831	277	3	Rab11-related AtRABA5a Rab11a
25	Solyc12g010790	Rab11	Solyc12g010790	12	-	2,364	654	218	2	Rab11-related AtRABA1f
26	Solyc12g014080	Rab11	Solyc12g014080	12	-	2,685	654	218	2	Rab11-related AtRABA1f Rab11B

Abbreviation used in Table3.21: Chr: Chromosome; Nt-Nucleotide sequence; Ex: No. of Exon,

The table 3.21 above shows the annotation of Tomato Rab11/ RabA Solyc Ids from SGN ITAG2.4. The 26 putative Rab11 GTPase annotated automatically are shown. The highlighted row with Solyc id: Solyc07g052290 is the target RabA1a (Rab11a) of the present study. Solyc10g050030 was not included in the present study because of short polypeptide of only 80 amino acid length.

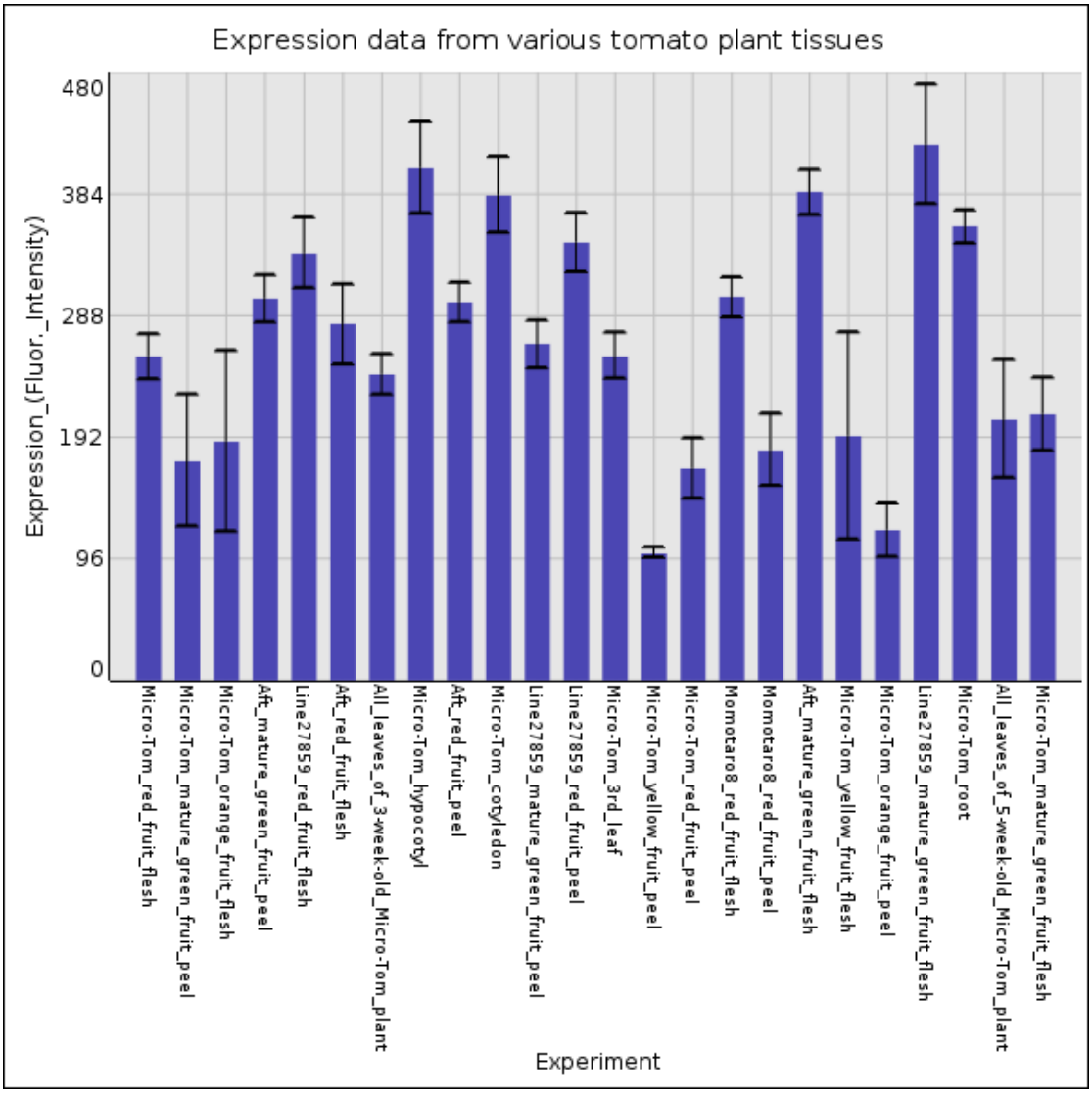


Fig.3.31 Expression of Solyc07g055290 (SolycRabA1a/Rab11a) from various tomato tissues

The fig. 3.31 above shows the digital expression of tomato RabA1a GTPase (Solyc07g055290) in the various tissues of tomato fruit including hypocotyl, mature green, red ripe, fruit peel and fruit flesh. The expression of Solyc07g05529 is found to be higher in hypocotyl, mature green fruit as compared to the red ripe fruit.

The MSA of the Rab11 GTPase with Solyc ids is shown in the fig. 3.32. The resulting phylogenetic tree of the tomato Rab11 GTPase is shown in fig. 3.33 where they fall in distinct sub groups. Major Clade of RabA1, A2, A4 and RabA5 sub group are shown. Tomato RabA1 has seven members, RabA2 has 6 members and Rab A4 and RabA5 have 5 members each. RabA3 and RabA6 both have one member each.

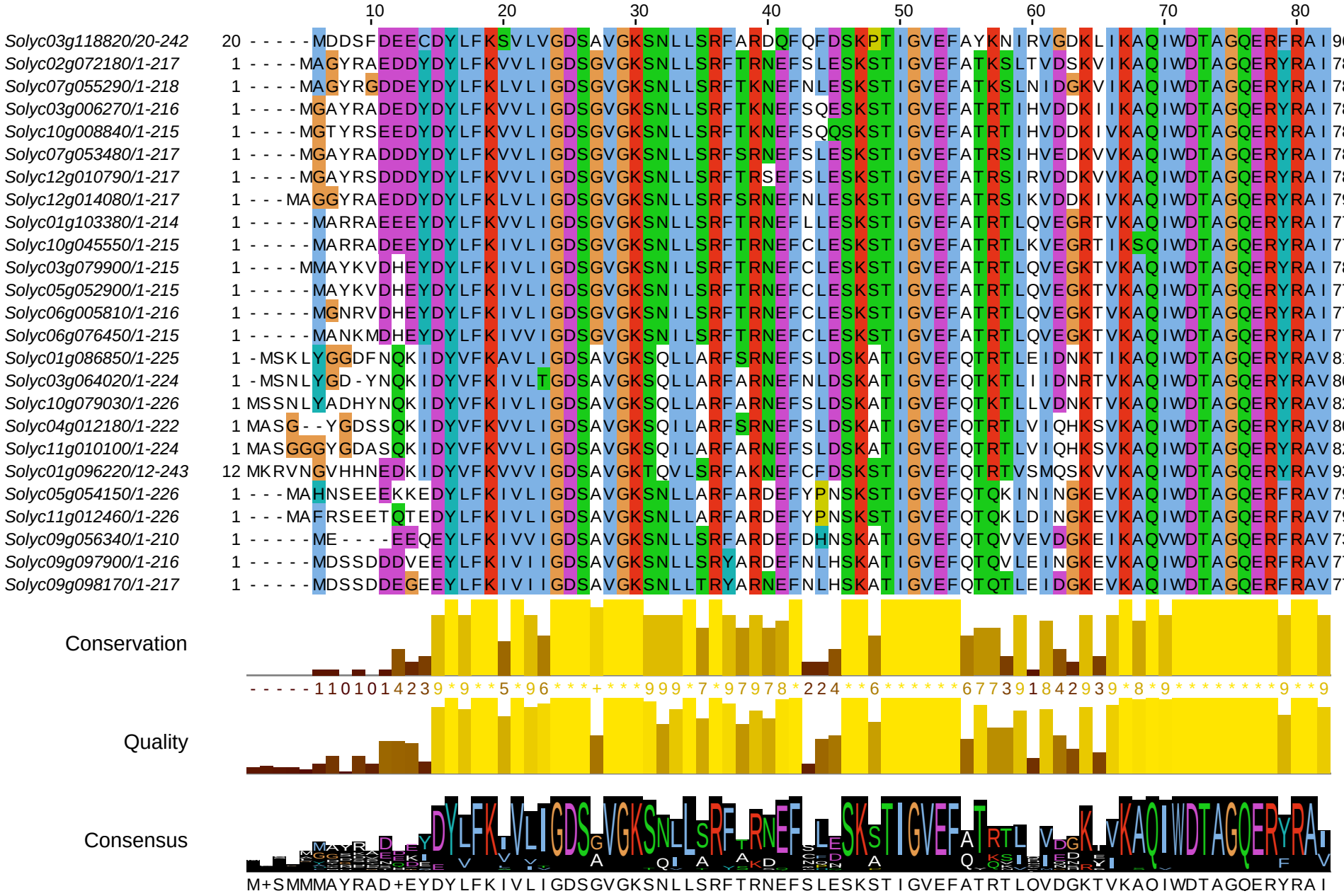


Fig.3.32 MSA of Tomato Rab11/RabA GTPase with Solyc Ids ver. ITAG2.4 generated by Jalview2.8.2 showing the group conservation plot, histogram, and consensus logo.

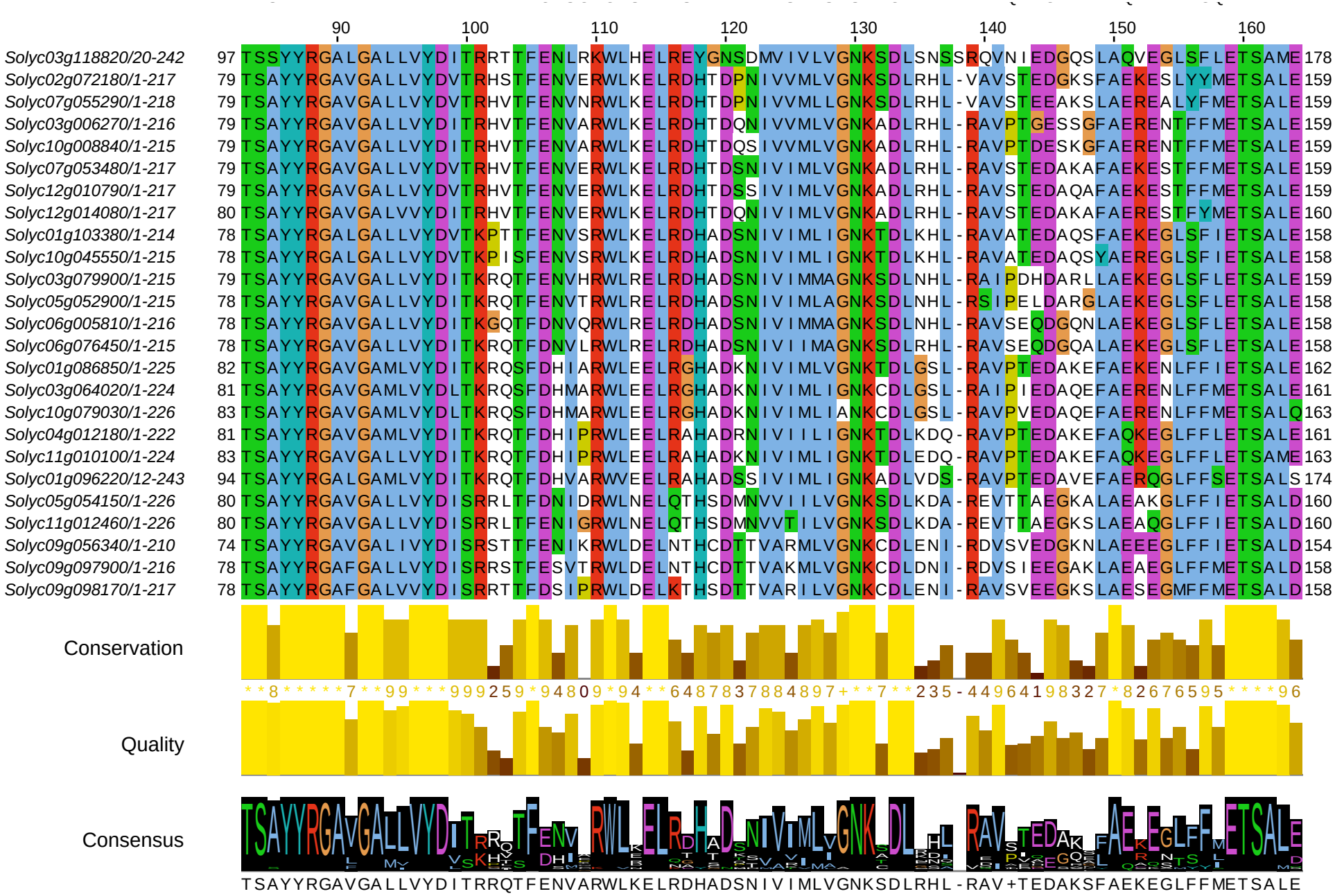


Fig. 3.32 MSA of Tomato Rab11/RabA GTPase with Solyc Ids ver. ITAG2.4 generated by Jalview2.8.2 showing the group conservation plot, histogram, and consensus logo.

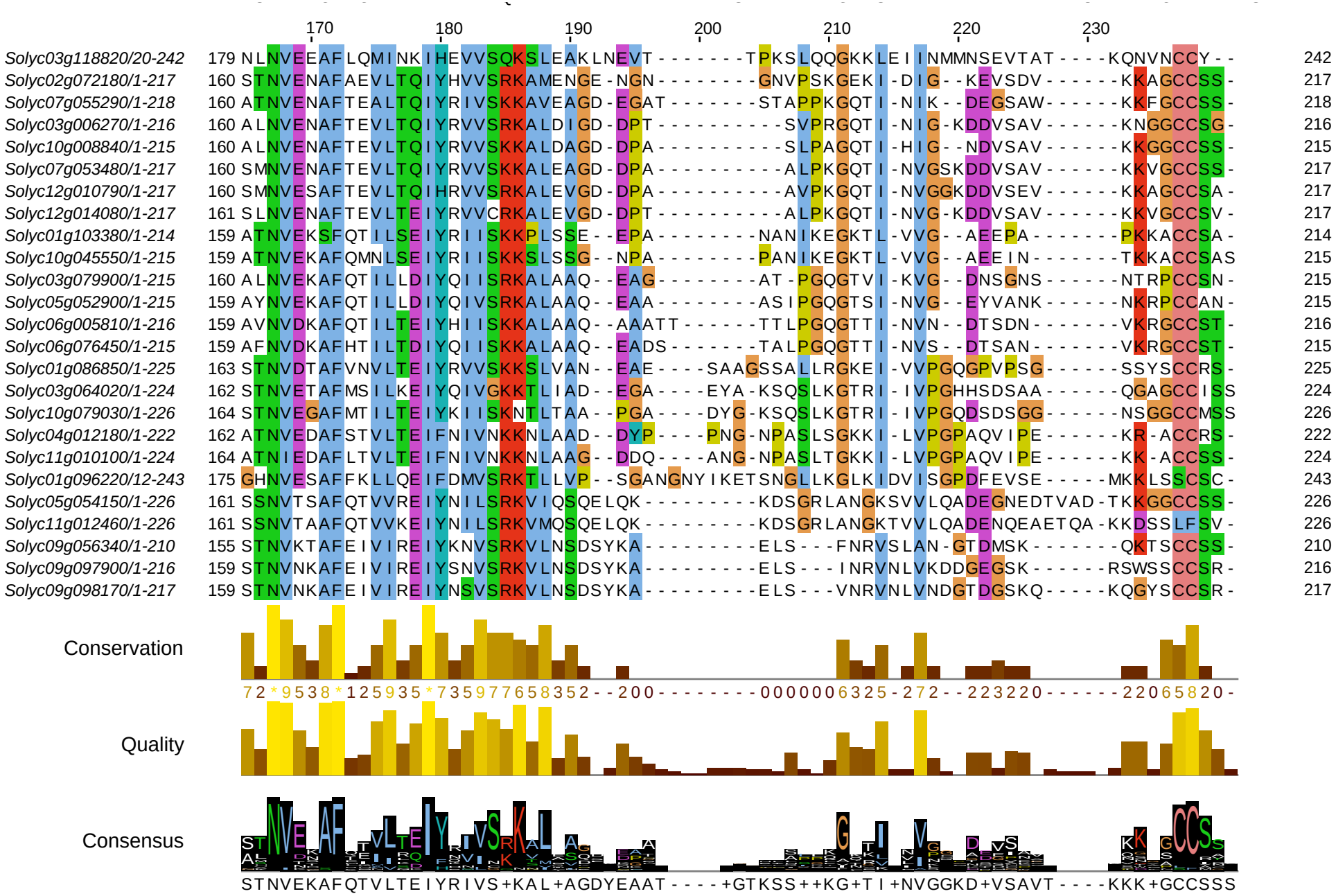


Fig. 3.32 MSA of Tomato Rab11/RabA GTPase with Solyc Ids ver. ITAG2.4 generated by Jalview2.8.2 showing the group conservation plot, histogram, and consensus logo.

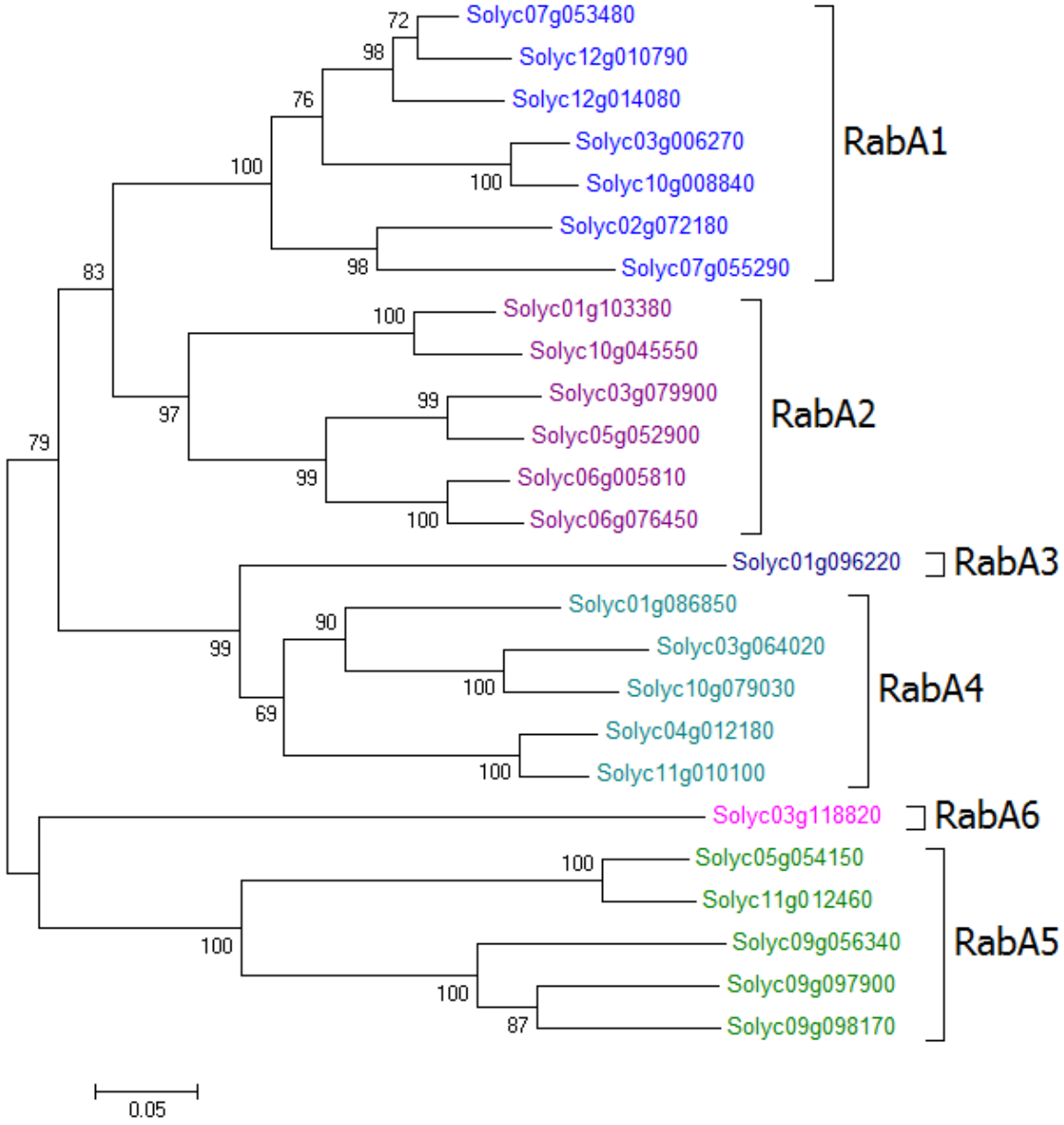


Fig. 3.33 Phylogenetic Tree of Tomato RabA/Rab 11 GTPase with SolyIds (ITAG2.4)

The phylogenetic tree was inferred using the Neighbor-Joining method (MEGA6). The optimal tree with the sum of branch length = 3.31 is shown. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Poisson correction method and are in the units of the number of amino acid substitutions per site. The analysis involved 25 tomato amino acid sequences. Seven tomato RabA fall in the cluster of RabA1 group, six in case of RabA2, followed by five each in the group of RabA4 and RabA5. Tomato RabA3 and tomato RabA6 had just one member in the class.

The verification of the domains of tomato RabA/Rab11 GTPase was carried out in SGN server. The protein sequences of the putative tomato Rab GTPases were used to generate the domain architecture profiles from the SGN database using the alignment analyzer feature. Fig.3.34 shows the domain architecture, a histogram of amino acid conservation and absolute coverage of the specific AA sequences. The pattern of the domains of the Rab GTPase is visible. Domain architecture helps in ascertaining the group of proteins falling and having same domain pattern characteristic of a protein.

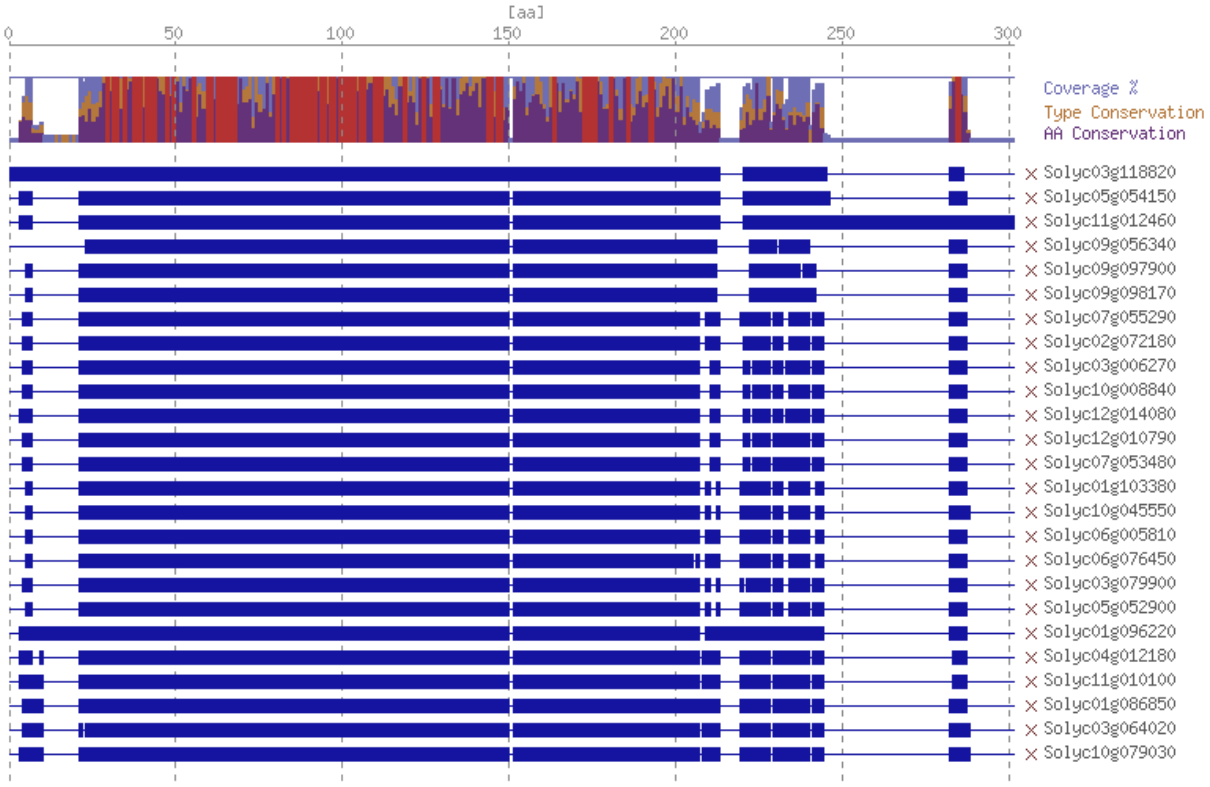


Fig. 3.34 Protein domains of the tomato Solyc Rab11/RabA GTPase.
The protein sequences were analysed to screen the domains of Rab11/RabA GTPase in the SGN server using the alignment analyzer tool with domain functionality.

The sequence Solyc10g050030 was not included in the analysis since it has incomplete ORF of only 80 amino acids. Solyc03g11820 a putative RabA6 and Solyc01g096220 were found to have elongated N-termini but otherwise had all the other Rab specific motifs and domain. Solyc11g012460 had an additional long C-terminus. Rest of the Solyc IDs qualified well with respective and complete domain architecture.

To validate the results the tomato RabA/Rab11GTPase were compared with the Arabidopsis RabA GTPase as shown in the MSA (fig.3.36). The phylogenetic tree of Tomato and Arabidopsis RabA GTPases (fig. 3.35) confirms the results and classification of the individual members of tomato RabA GTPase.

Clade1- RabA1: Clade1 consists of RabA1 sub group with 7 tomato RabA1 GTPase falling in the same cluster with nine of Arabidopsis RabA1. Tomato RabA1a (Solyc07g055290) falls together with AtRabA1a. Solyc02g072180 falls in same node with the AtRabA1B and AtRabA1D. Solyc12g010790, Solyc12g014080 and Solyc07g053480 fall together with AtRabA1f. Rest of the two tomato sequence Solyc03g006270 and Solyc10g008840 lie in node with AtRabA1e, h and AtRabA1i.

Clade2- RabA2: Clade2 consists of RabA2 sub group with six of tomato RabA2 falling in the same cluster with four of the Arabidopsis RabA2 GTPase. RabA2a of Arabidopsis has two members of tomato viz. Solyc01g103380 and Solyc10g045550. Other four tomato RabA2 members (Solyc06g05810, Solyc06g076450, Solyc03g079900 and Soly05g052900) fall together with AtRabA2b, c and AtRabA2d.

Clade3- RabA5: Clade3 consists of RabA5 sub group with five of tomato RabA5 falling in the same cluster with five of Arabidopsis RabA5. AtRabA5a has two members in tomato *viz.* Solyc05g054150 and Solyc11g012460. Solyc09g056340 falls in the same node with AtRabA5b. Other two members of tomato RabA5 (Solyc09g097900 and Solyc09g098170) fall in line with At RabA5C, D and AtRabA5E.

Clade4- RabA6: Clade 4 consists of RabA6 sub group with one member of tomato RabA6 (Soly03g118820) falling in the same cluster with the two Arabidopsis RabA6 *i.e* AtRabA6a and AtRabA6b.

Clade5- RabA4: Clade 5 consists of RabA4 sub group with five of tomato RabA4 falling in the same cluster with four of Arabidopsis RabA4. Solyc04g012180 and Solyc11g010100 fall together with AtRabA4a and AtRabA4b. Solyc01g086850 falls together with AtRabA4c whereas; At RabA4d has two members of tomato Solyc03g064020 and Solyc10g079030 in the same cluster.

Clade6- RabA3: Clade 6 consists of RabA3 sub group with one member of tomato RabA3 (Solyc01g096220) falling in the same node with the Arabidopsis AtRabA3.

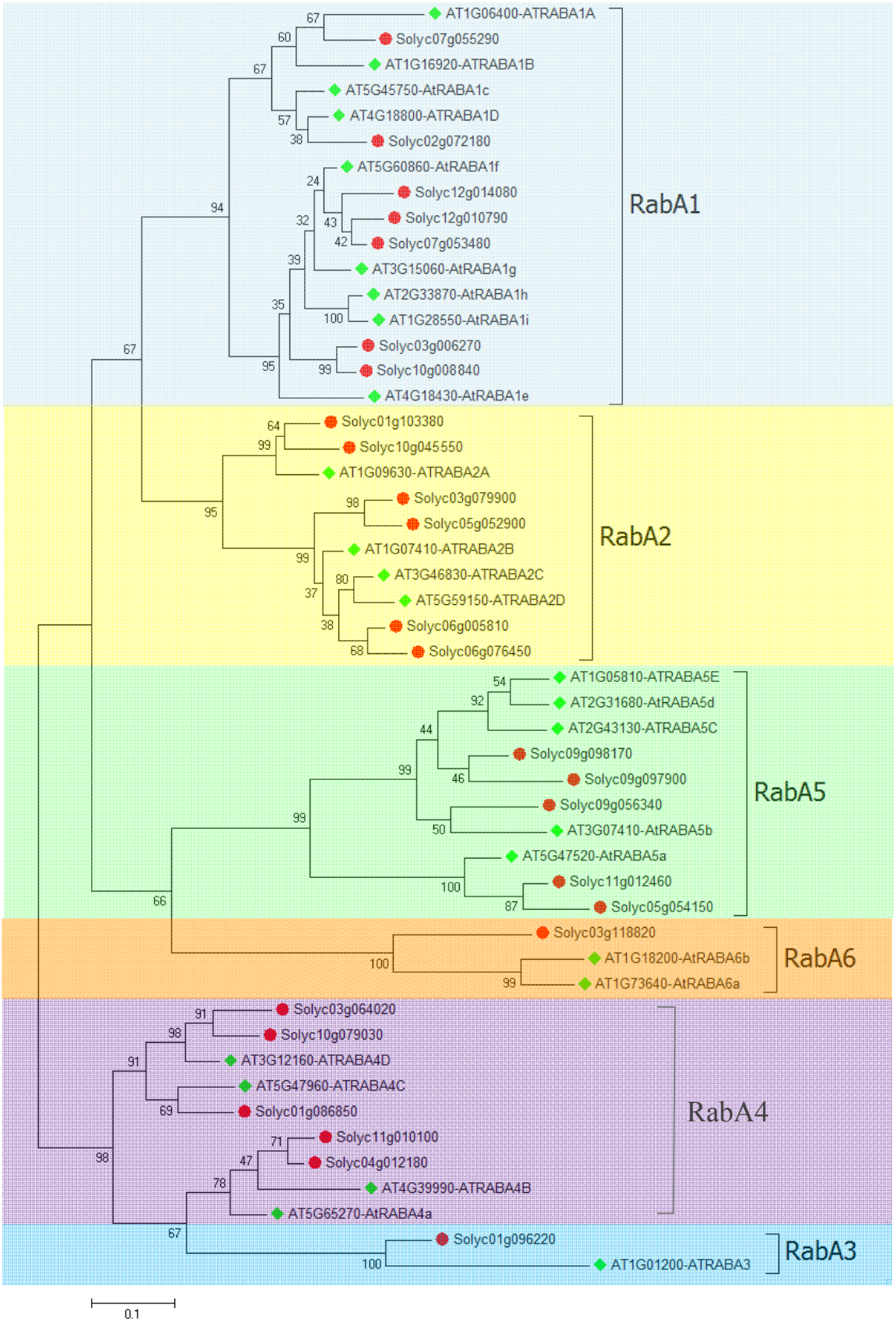


Fig. 3.35 Phylogenetic Tree of Arabidopsis and Tomato RabA/Rab11 GTPase with SolyIds (ITAG2.4). The neighbour joining phylogenetic tree with 1000 boot strap value is shown. Each tomato RabA GTPase falls in the distinct sub group of the Arabidopsis RabA GTPase. The node markers with red circle and green diamond show tomato and Arabidopsis sequence ids respectively. For description please see text.

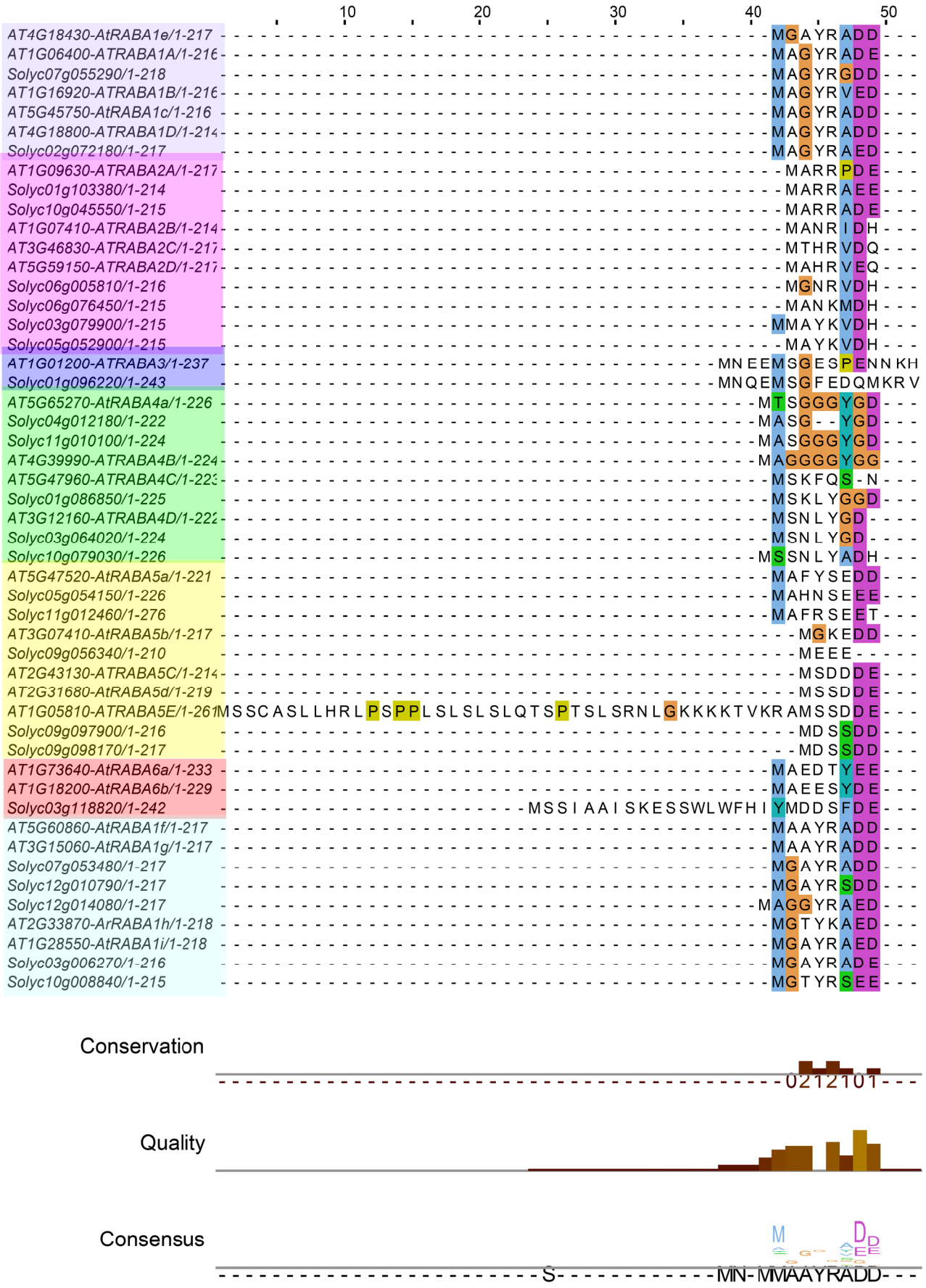


Fig.3.36 MSA of Arabidopsis and Tomato Rab11/RabA GTPase with Solyc Ids ver. ITAG2.4 generated by Jalview2.8.2 showing the group conservation plot, histogram, and consensus logo.



Fig. 3.36 MSA of Arabidopsis and Tomato Rab11/RabA GTPase with Solyc Ids ver. ITAG2.4 generated by Jalview2.8.2 showing the group conservation plot, histogram, and consensus logo.

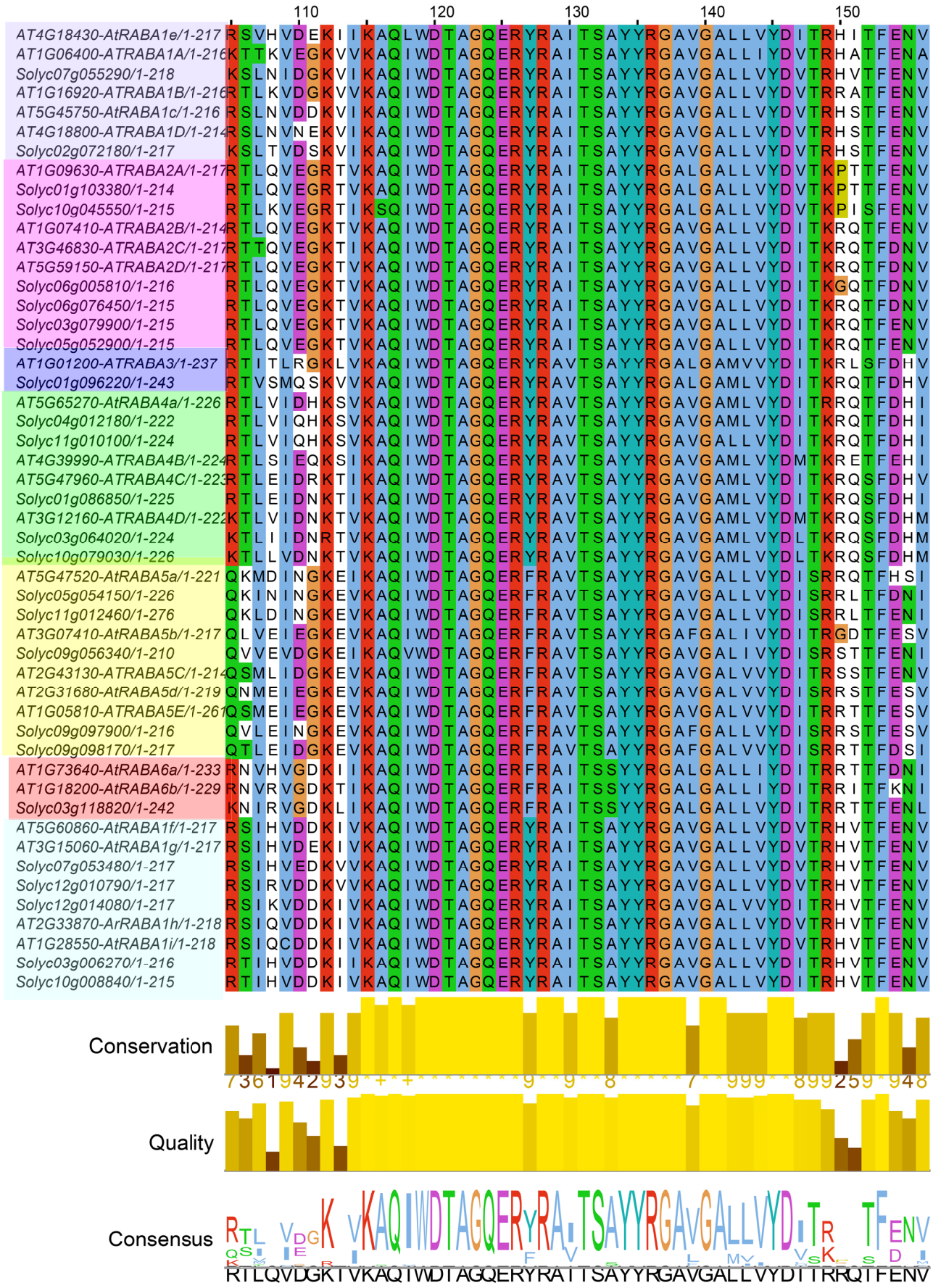


Fig.3.36 MSA of Arabidopsis and Tomato Rab11/RabA GTPase with Solyc Ids ver. ITAG2.4 generated by Jalview2.8.2 showing the group conservation plot, histogram, and consensus logo.



Fig.3.36 MSA of Arabidopsis and Tomato Rab11/RabA GTPase with Solyc Ids ver. ITAG2.4 cont...

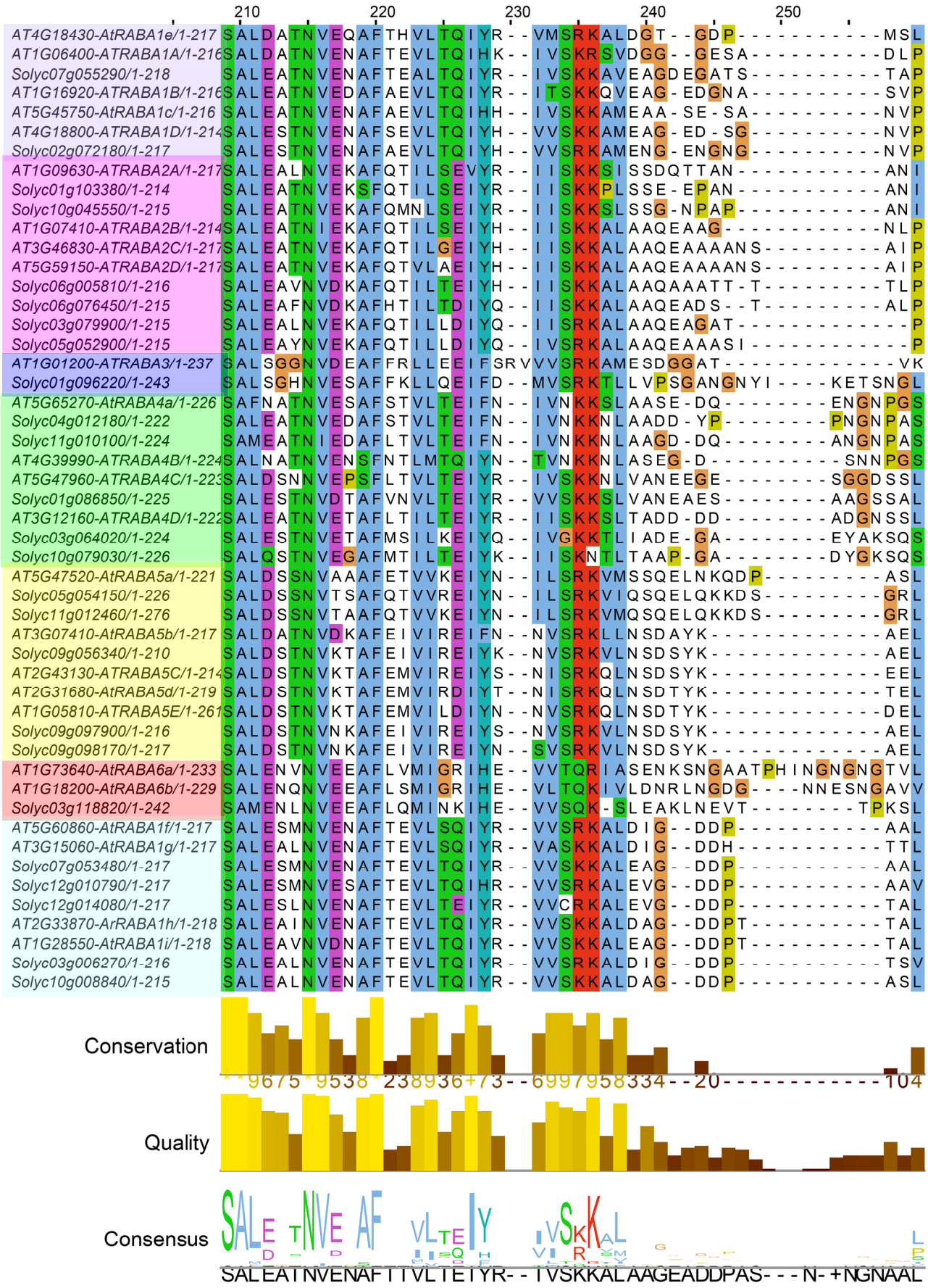


Fig.3.36 MSA of Arabidopsis and Tomato Rab11/RabA GTPase with Solyc Ids ver. ITAG2.4 cont...

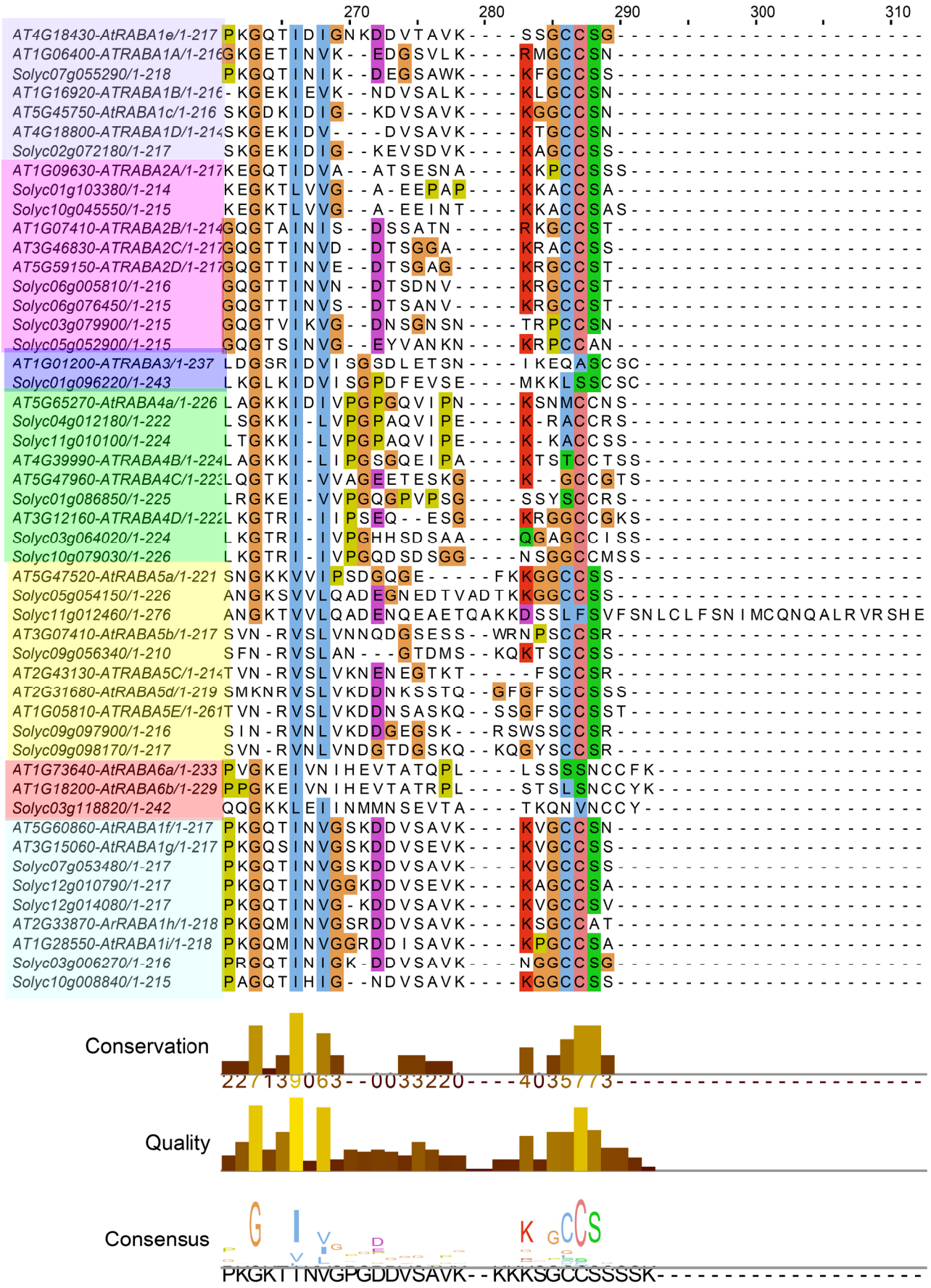


Fig.3.36 MSA of Arabidopsis and Tomato Rab11/RabA GTPase with Solyc Ids ver. ITAG2.4 cont...

Table 3.22 List of Tomato Rab GTPase (Rab1, 2, 5, 6, 7, 8 and Rab18) Solyc Ids from SGN ITAG2.4 Release with sequence id, strand, Nucleotide sequence, mRNA and amino acid sequence length.

Sr. No.	Rab	Locus symbol	Chr	Annotation	Locus name	Region	Strand	NT	mRNA	Protein
	Rab1	**/RabD**								
1	Rab1	Solyc01g090170	1	Ras-related protein Rab-1A	Solyc01g090170.2	SL2.50ch01:83802202..83809193	+	6992	1,039	204
2	Rab1	Solyc01g103370	1	rab1b LeRab1B	Solyc01g103370.2	SL2.50ch01:91989852..91994839	+	4988	969	203
3	Rab1	Solyc05g052070	5	rab1c LeRab1C	Solyc05g052070.2	SL2.50ch05:62410368..62413664	-	3,297	1,159	204
4	Rab1	Solyc05g053940	5	Ras-related protein Rab-1A	Solyc05g053940.2	SL2.50ch05:63958473..63963310	-	4838	1,085	204
5	Rab1	Solyc08g078070	8	rab1a LeRab1A	Solyc08g078070.2	SL2.50ch08:61918544..61921746	+	3203	1,044	204
	Rab2	**/RabB**								
1	Rab2	Solyc02g093530	2	Ras-related protein Rab-2-A	Solyc02g093530.2	SL2.50ch02:54369660..54374256	-	4,597	1,082	212
2	Rab2	Solyc06g060210	6	Ras-related protein Rab-2-A	Solyc06g060210.1	SL2.50ch06:38174983..38175594	+	612	612	204
3	Rab2	Solyc07g056150	7	Ras-related protein Rab-2-A	Solyc07g056150.2	SL2.50ch07:63994885..63999176	-	4,292	1,897	213
4	Rab2	Solyc10g007700	10	Ras-related protein Rab-2-A	Solyc10g007700.2	SL2.50ch10:1953681..1958548	-	4,868	979	212
5	Rab2	Solyc12g011130	12	Ras-related protein Rab-2-A	Solyc12g011130.1	SL2.50ch12:3971227..3974056	-	2,830	636	212

	Rab5	**/RabF**								
Sr. No.	**Rab**	**Locus symbol**	**Chr**	**Annotation**	**Locus name**	**Region**	**Strand**	**NT**	**mRNA**	**Protein**
1	Rab 5	Solyc02g036450	2	Ras-related protein Rab-5C	Solyc02g036450.2	SL2.50ch02:30788590..30794213	-	5,624	1,062	201
2	Rab 5	Solyc02g069370	2	Ras-related protein Rab-5C	Solyc02g069370.2	SL2.50ch02:39281375..39289536	-	8,162	1,016	201
3	Rab 5	Solyc02g081380	2	Ras-related protein Rab-5C	Solyc02g081380.2	SL2.50ch02:45368874..45373407	-	4,534	1,134	201
4	Rab 5	Solyc05g045660	5	Ras-related protein Rab-5C	Solyc05g045660.2	SL2.50ch05:57401079..57403786	+	2,708	963	213
5	Rab 5	Solyc11g008430	11	Ras-related protein Rab-5C	Solyc11g008430.1	SL2.50ch11:2614324..2620969	-	6,646	603	201
6	Rab 5	Solyc12g005840	12	Ras-related protein Rab-5C	Solyc12g005840.1	SL2.50ch12:474797..477406	-	2,610	903	301

Sr. No.	Rab	Locus symbol	Chr	Annotation	Locus name	Region	Strand	NT	mRNA	Protein
1	Rab6	Solyc01g088560	1	Ras-related protein Rab-6A	Solyc01g088560.2	SL2.50ch01:83336020..83341655	-	5,636	1,147	209
2	Rab6	Solyc03g078570	3	Ras-related protein Rab-6A	Solyc03g078570.2	SL2.50ch03:51178678..51181240	-	2,563	1,184	208
3	Rab6	Solyc05g051570	5	Ras-related protein Rab-6A	Solyc05g051570.2	SL2.50ch05:61938794..61942841	-	4,048	1,121	208
	Rab7	**/RabG**								
1	Rab7	Solyc01g109520	1	Ras-related protein Rab-7	Solyc01g109520.2	SL2.50ch01:96449221..96452841	-	3,621	1,080	207
2	Rab7	Solyc03g120750	3	Ras-related protein Rab-7	Solyc03g120750.2	SL2.50ch03:69027066..69031274	-	4,209	840	206
3	Rab7	Solyc04g051680	4	Ras-related protein Rab-7	Solyc04g051680.2	SL2.50ch04:50982372..50985730	-	3,359	856	220
4	Rab7	Solyc04g072060	4	Ras-related protein Rab-7a	Solyc04g072060.2	SL2.50ch04:59116633..59126825	-	10,193	1,025	205
	Rab8	**/RabE**								
Sr. No.	**Rab**	**Locus symbol**	**Chr**	**Annotation**	**Locus name**	**Region**	**Strand**	**NT**	**mRNA**	**Protein**
1	Rab8	Solyc04g011360	4	Ras-related protein Rab-8A	Solyc04g011360.2	SL2.50ch04:3839267..3844110	-	4,844	1,200	217
2	Rab8	Solyc06g005350	6	Ras-related protein Rab-8A	Solyc06g005350.2	SL2.50ch06:362156..370204	-	8,049	612	145
3	Rab8	Solyc09g010370	9	Ras-related protein Rab-8A	Solyc09g010370.2	SL2.50ch09:3753893..3759018	-	5,126	1,050	217
4	Rab8	Solyc10g086350	10	Ras-related protein Rab-8A	Solyc10g086350.1	SL2.50ch10:65205566..65210133	-	4568	651	217
5	Rab8	Solyc11g073050	11	Ras-related protein Rab-8A	Solyc11g073050.1	SL2.50ch11:56164984..56168738		3,755	651	217
	Rab18	**/RabC**								
1	Rab18	Solyc04g064510	4	Rab-18 AtRABC1, AtRab18	Solyc04g064510.2	SL2.50ch04:55670912..55675897	-	4,986	1,084	210
2	Rab18	Solyc07g052410	7	Ras-related protein Rab-18	Solyc07g052410.2	SL2.50ch07:60927071..60929129	+	2,059	988	258
3	Rab18	Solyc09g008460	9	Rab-18 AtRab18B, AtRABC2a	Solyc09g008460.2	SL2.50ch09:1905656..1911197	-	5542	1,202	217
4	Rab18	Solyc10g086310	10	Ras-related protein Rab-18	Solyc10g086310.1	SL2.50ch10:65177681..65179081	-	1,401	630	210
5	Rab18	Solyc11g008020	11	Ras-related protein Rab-18	Solyc11g008020.1	SL2.50ch11:2238213..2241626	+	3414	651	217
	Miro	**GTPase**								
1	Miro	Solyc04g051700	4	Ras-related protein RAB-4 Miro-like	Solyc04g051700.2	SL2.50ch04:51022896..51030005	+	7,110	1,327	334
2	Miro	Solyc07g064290	7	Ras-related protein Rab-21 Miro-like	Solyc07g064290.2	SL2.50ch07:66496422..66500636	-	4,215	1,340	378

Table 3.22 shows the list of Tomato Rab 1, 2, 5, 6, 7, 8 and Rab18 GTPase Solyc Ids from SGN (ITAG2.4) with sequence ids, strand, nucleotide sequence, mRNA and amino acid sequence length. The retrieved sequences were used to construct MSA for tomato and Arabidopsis Rab GTPase (Fig.3.37). Phylogenetic tree of 30 protein sequences of tomato Rab1, 2, 5, 6, 7, 8 and Rab18 (Fig3.38) revealed that the individual tomato Rab GTPase fall in the same cluster of their respective groups. Clade1 Consists of five tomato Rab1/RabD members; Clade2 Consist of five Rab8/RabE; Clade3 consists of 4 tomato Rab18/RabC GTPase; Clade4 consist of five members of Rab2/RabB; Clade5 consist of four tomato Rab5/RabF; Clade6 consists of three Rab6/RabH GTPase and the last Clade7 consist of four tomato Rab7/RabG GTPase.

In order to validate the results the tomato Rab GTPase protein sequences were aligned with Arabidopsis for MSA and phylogenetic tree was constructed to test the classification. The MSA (Fig.3.39) and phylogenetic tree of Arabidopsis and Tomato Rab1, 2, 5, 6, 7, 8 and Rab18 (Fig. 3.40) confirms the results and classification as discussed below

Clade1: The five genes of tomato Rab8/RabE GTPase (Solyc04g011360, Solyc06g005350, Solyc09g010370, Solyc10g086350 and Solyc11g073050) fall together with 5 At Rab8/RabE.

Clade2: There are five members of tomato Rab1/RabD (Solyc01g090170, Solyc01g103370, Solyc05g052070, Solyc05g053940 and Solyc08g078070) falling in the same cluster with four AtRab1/RabD GTPase.

Clade3: The four members of tomato Rab18/RabC (Solyc04g064510, Solyc09g008460, Solyc10g086310 and Solyc11g008020) fall in cluster with three Arabidopsis AtRab18/C. The sequence Solyc07g052410 was not included in the analysis.

Clade4: Five members of tomato Rab2/RabB GTPase (Solyc02g093530, Solyc06g060210, Solyc07g056150, Solyc10g007700 and Solyc12g011130) are clustered together in the same group with four of Arabidopsis Rab2 GTPase.

Clade5: Three members of Arabidopsis and four members of Tomato Rab5/RabE GTPase (Solyc02g036450, Solyc02g069370, Solyc02g081380 and Solyc11g008430) fall in the same cluster. Solyc12g005840 and Solyc05g045660 were not included in the present analysis

Clade6: Five of AtRab6 fall together in the same cluster with three members of tomato Rab6/RabH (Solyc01g088560, Solyc03g078570 and Solyc05g051570).

Clade7: Four members of tomato Rab7/RabG (Solyc01g109520, Solyc03g120750, Solyc04g051680 and Solyc04g072060) fall in the same cluster with seven members of AtRab7/RabG GTPase. The above results confirm and validate the classification and grouping of the current tomato Rab GTPases.

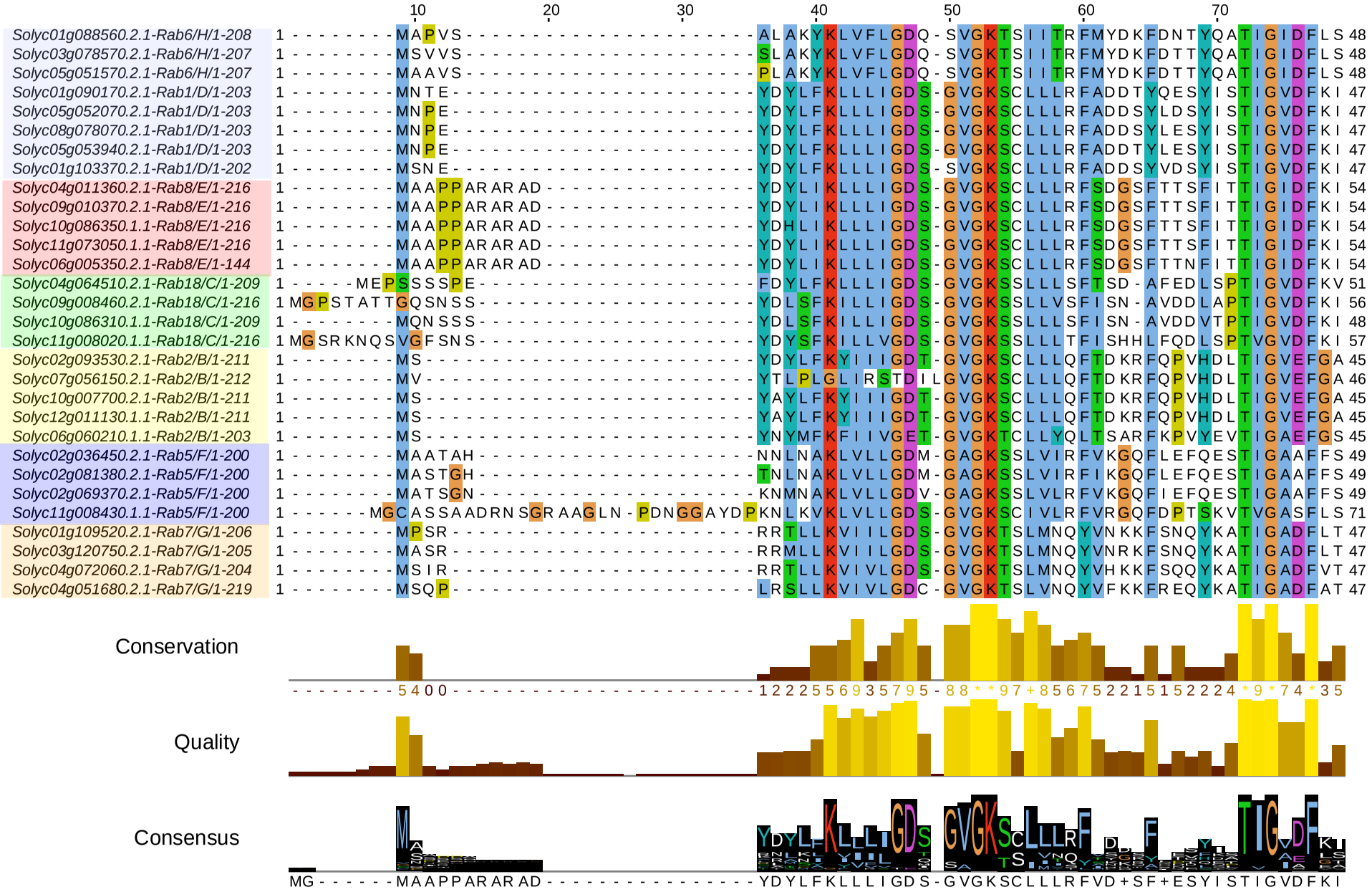


Fig.3.37 MSA of Tomato Rab1,2,5,6,7,8 and Rab18 GTPase with Solyc Ids ver. ITAG2.4 generated by Jalview2.8.2 showing the group conservation plot, histogram, and consensus logo.

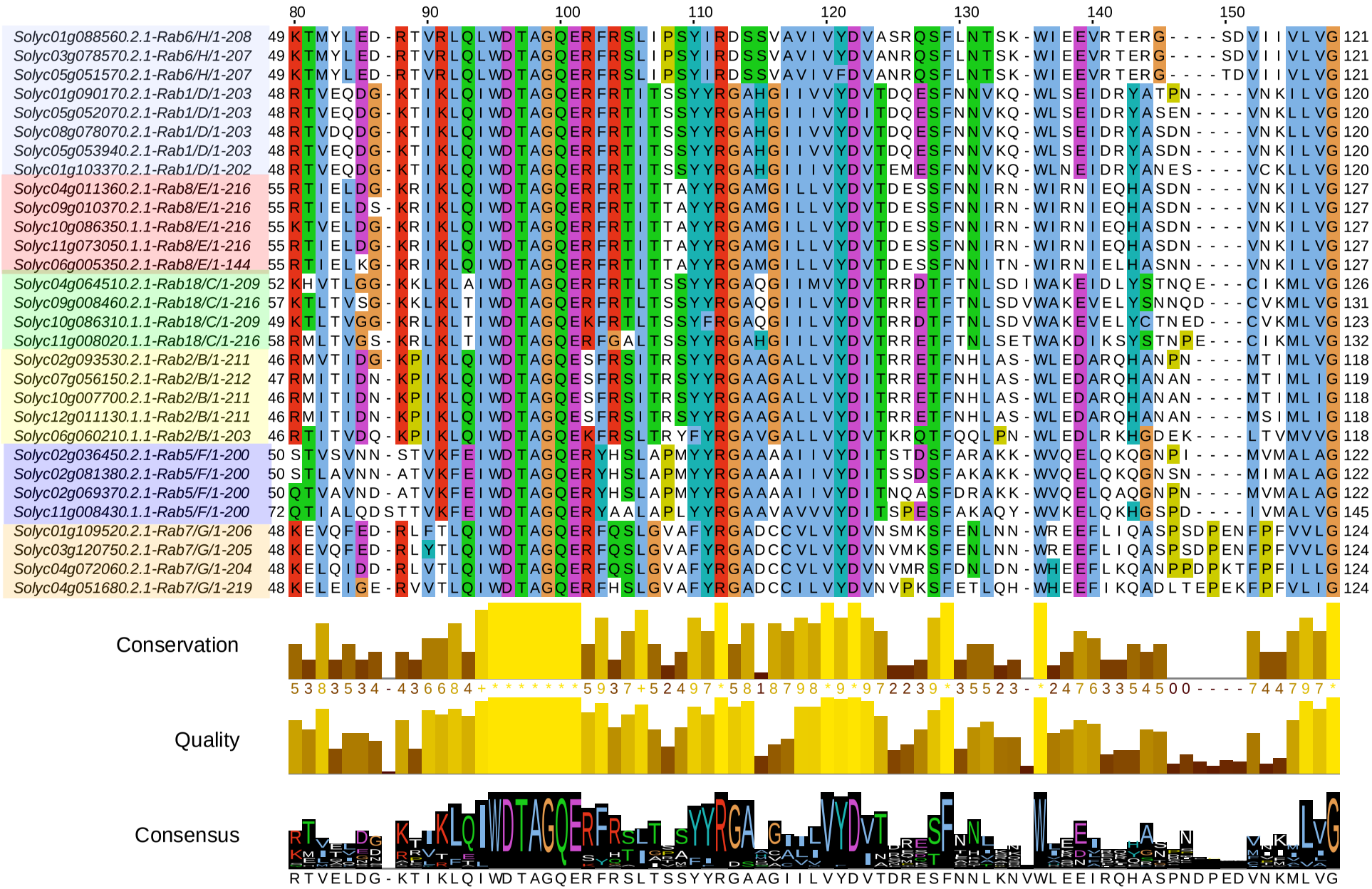


Fig.3.37 MSA of Tomato Rab1,2,5,6,7,8 and Rab18 GTPase with Solyc Ids ver. ITAG2.4 cont...

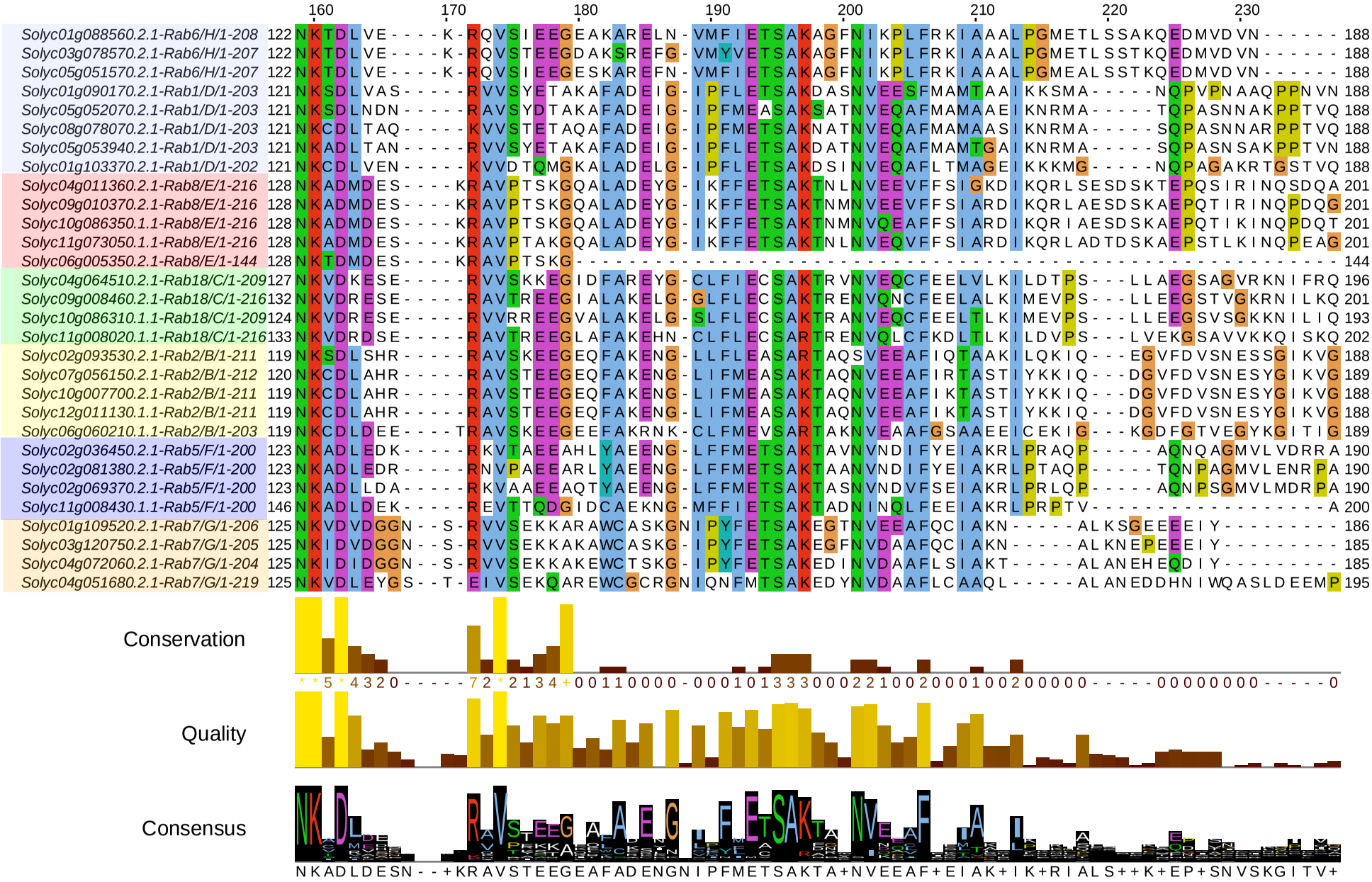


Fig.3.37 MSA of Tomato Rab1,2,5,6,7,8 and Rab18 GTPase with Solyc Ids ver. ITAG2.4 cont...

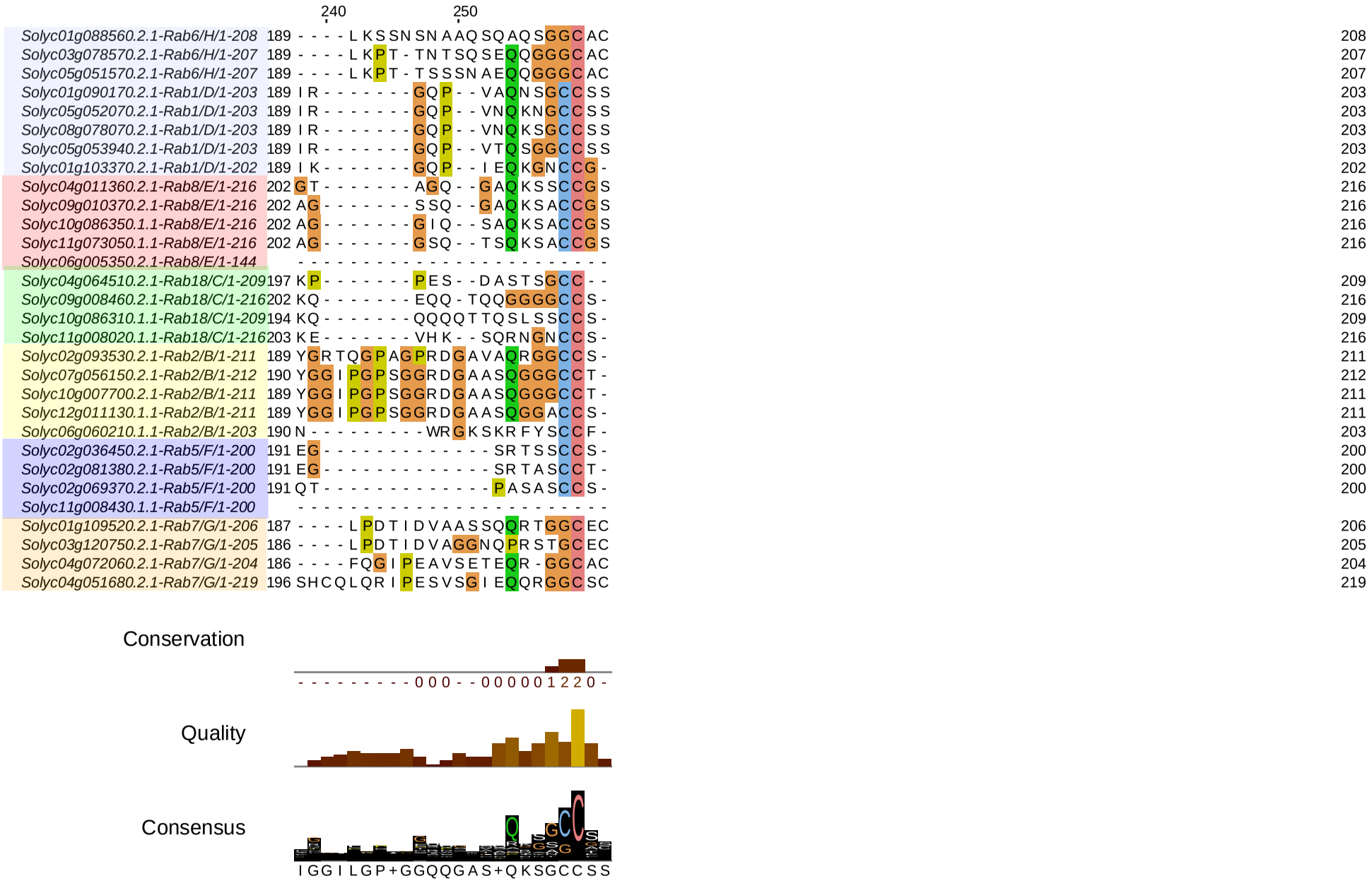


Fig.3.37 MSA of Tomato Rab1,2,5,6,7,8 and Rab18 GTPase with Solyc Ids ver. ITAG2.4

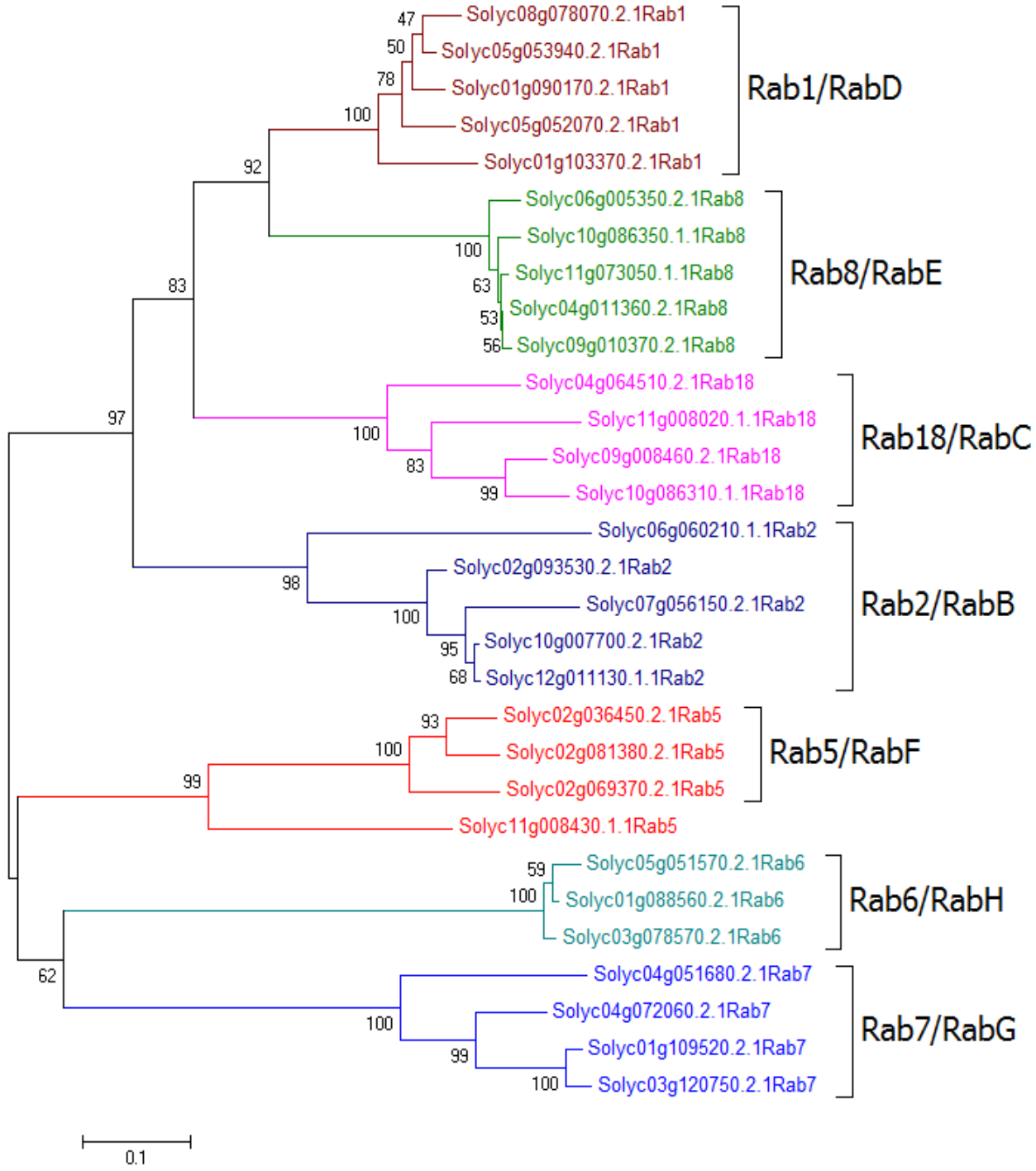


Fig. 3.38 Phylogenetic tree of Tomato Solyc Rab1, 2, 5, 6, 7, 8 and Rab18 (SGN- ITAG2.4)
The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 4.31 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Poisson correction method and are in the units of the number of amino acid substitutions per site. The analysis involved 30 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 131 positions in the final dataset. Evolutionary analyses were conducted in MEGA6.

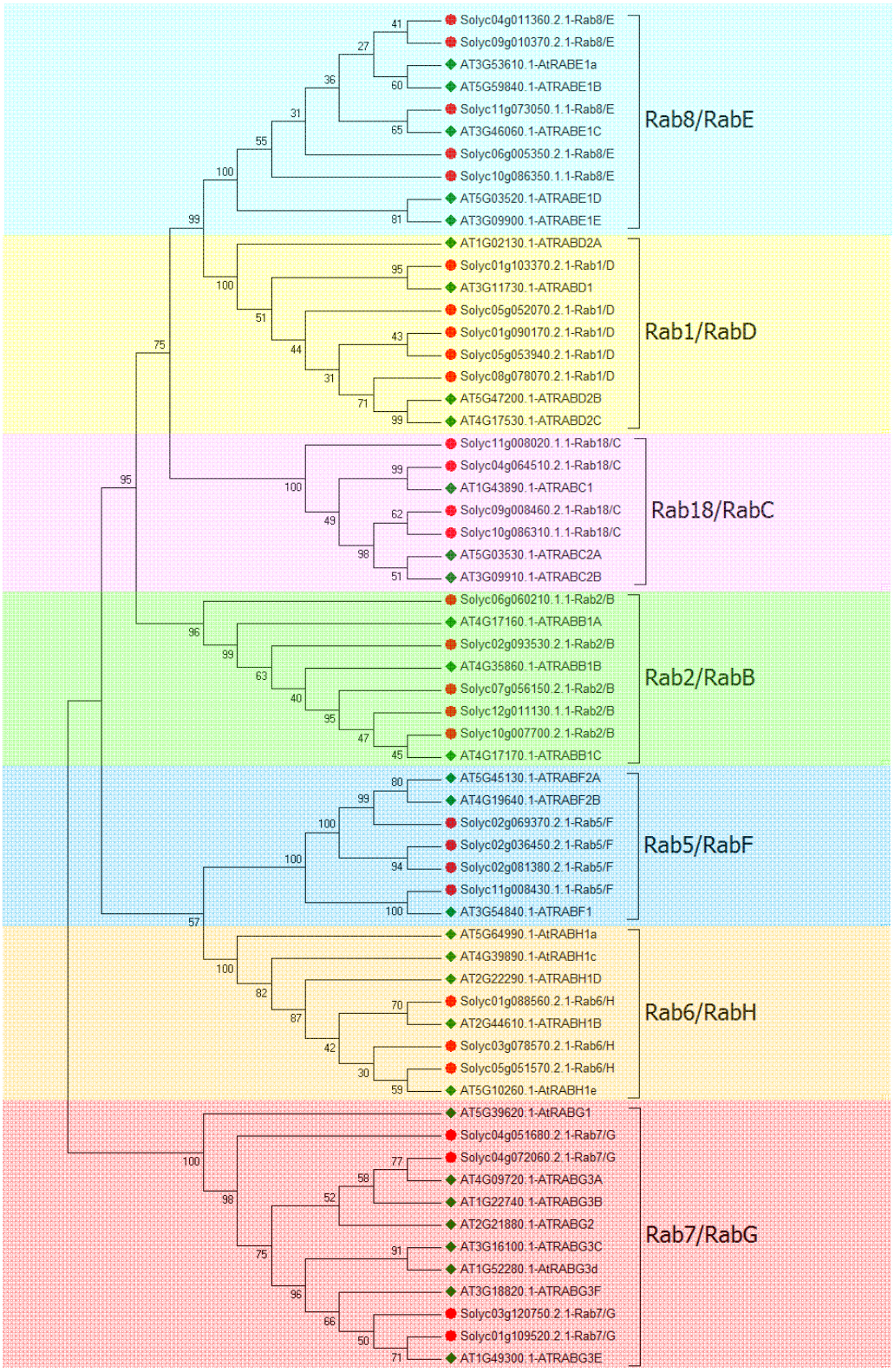


Fig. 3.39 Phylogenetic tree of Arabidopsis and Tomato Solyc Rab1, 2, 5, 6, 7, 8 and Rab18 (SGN-ITAG2.4). The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 7.19 is shown. The percentage of replicate trees with the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Poisson correction method and are in the units of the number of amino acid substitutions per site. The analysis involved a total of 61 amino acid sequences including 31 from Arabidopsis and 30 from Tomato. The node markers with red circle and green diamond show tomato and Arabidopsis sequence ids respectively. For convenience the tree represents the topology mode only. Evolutionary analyses were conducted in MEGA6.

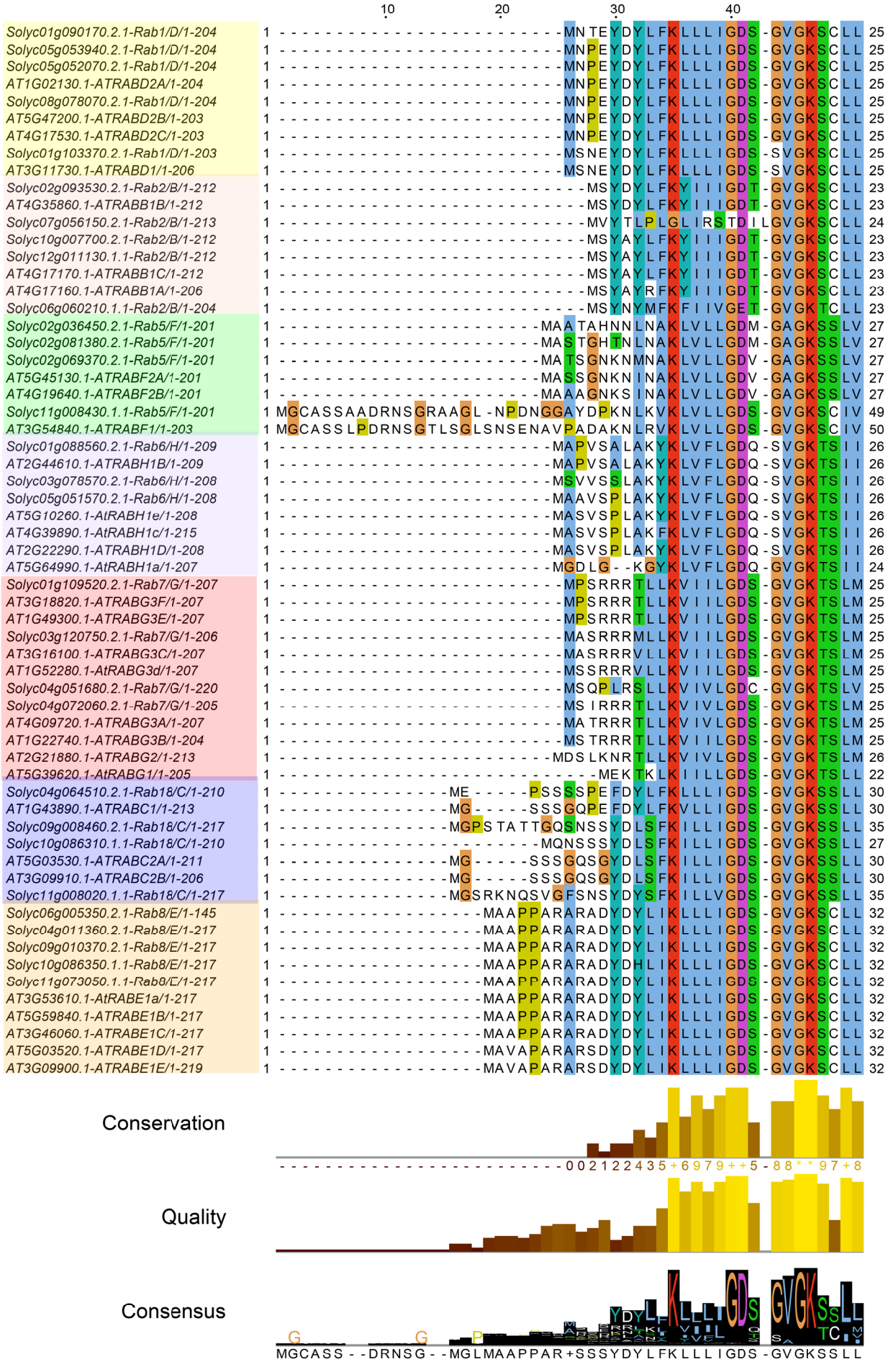


Fig.3.40 MSA of Arabidopsis and Tomato Rab1,2,5,6,7,8 and Rab18 GTPase with Solyc Ids ver. ITAG2.4 generated by Jalview2.8.2 showing the group conservation plot, histogram, and consensus logo.

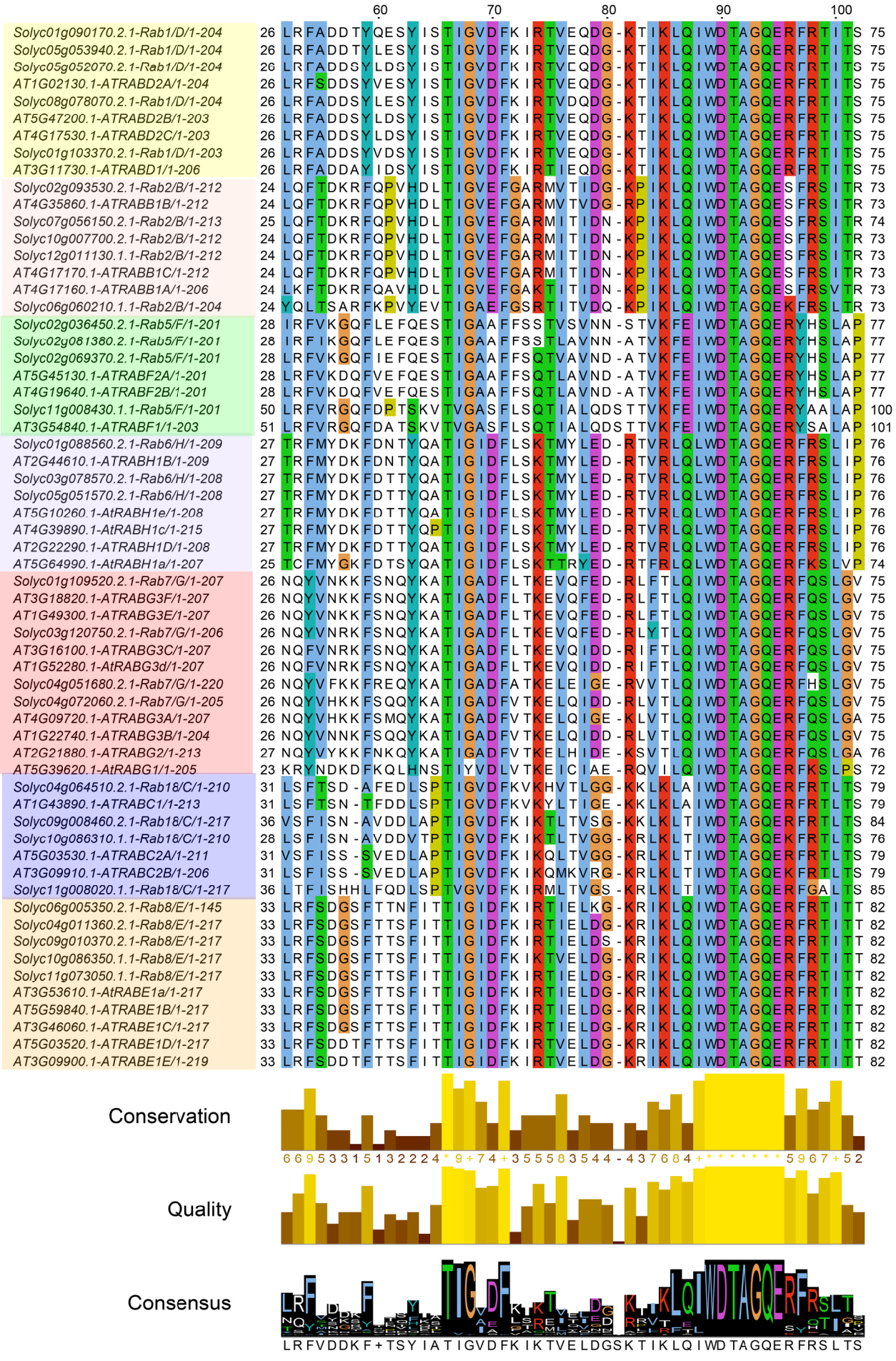


Fig.3.40 MSA of Arabidopsis and Tomato Rab1,2,5,6,7,8 and Rab18 GTPase cont...

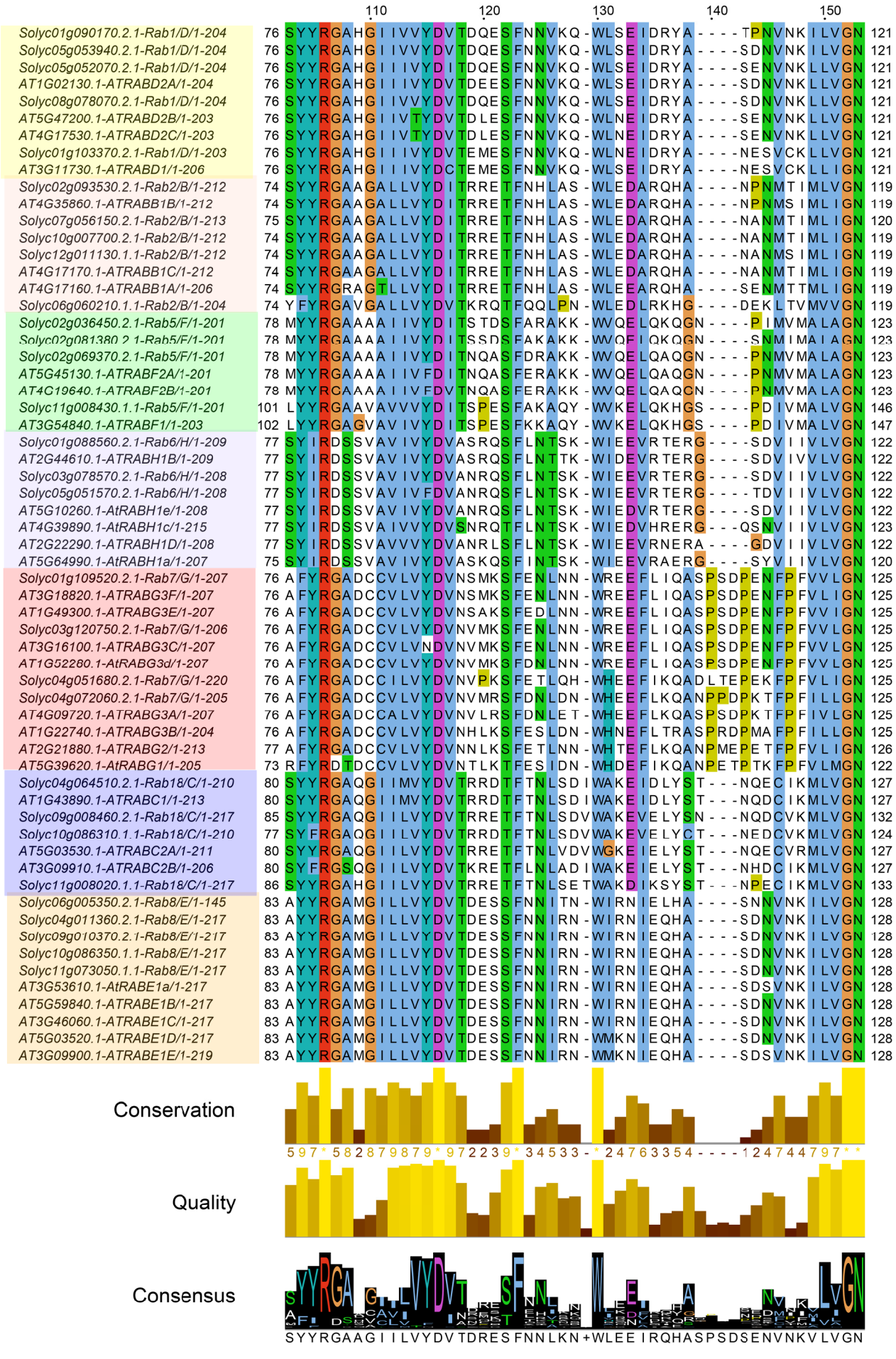


Fig.3.40 MSA of Arabidopsis and Tomato Rab1,2,5,6,7,8 and Rab18 GTPase cont...

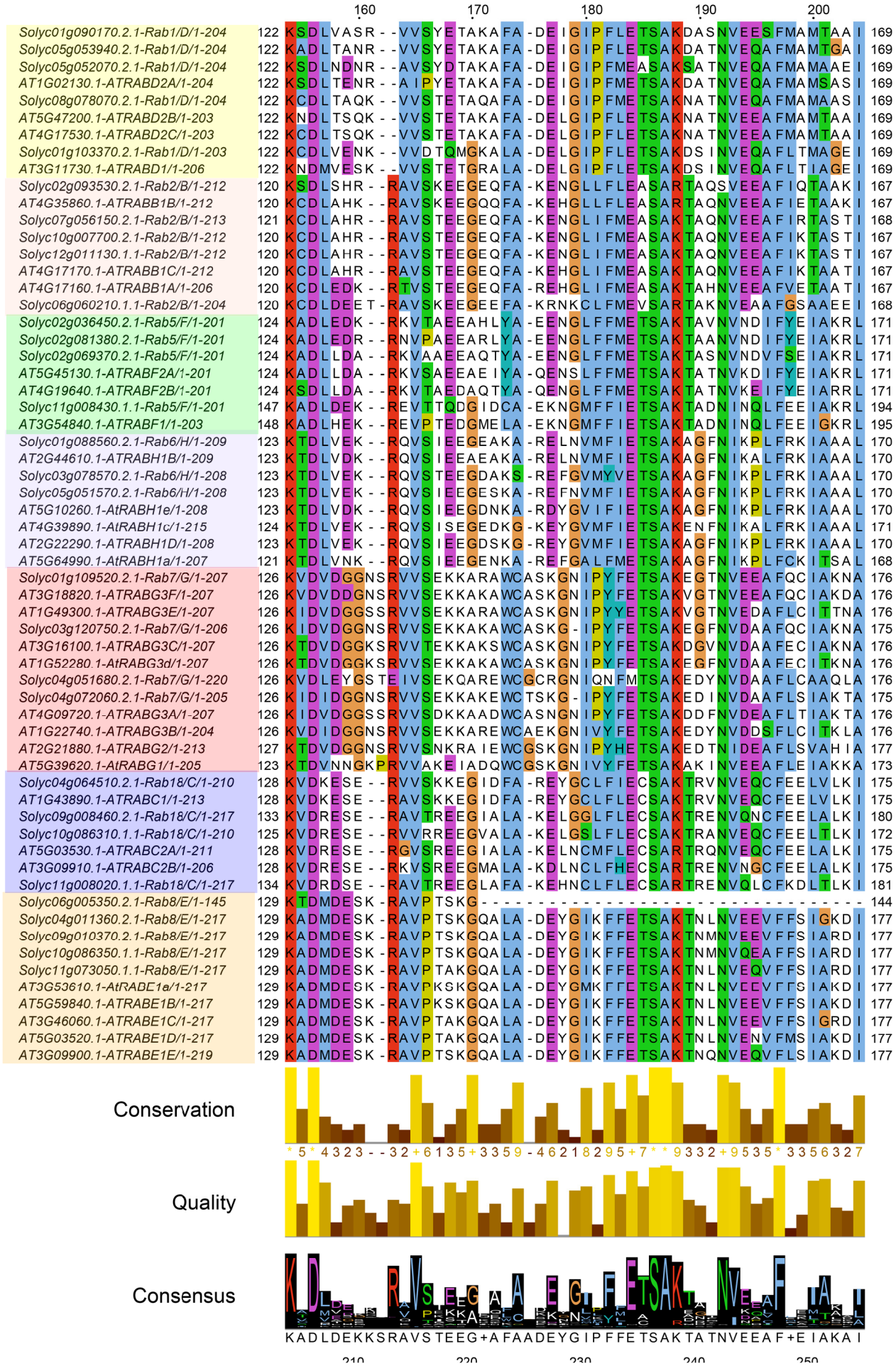


Fig.3.40 MSA of Arabidopsis and Tomato Rab1,2,5,6,7,8 and Rab18 GTPase cont...

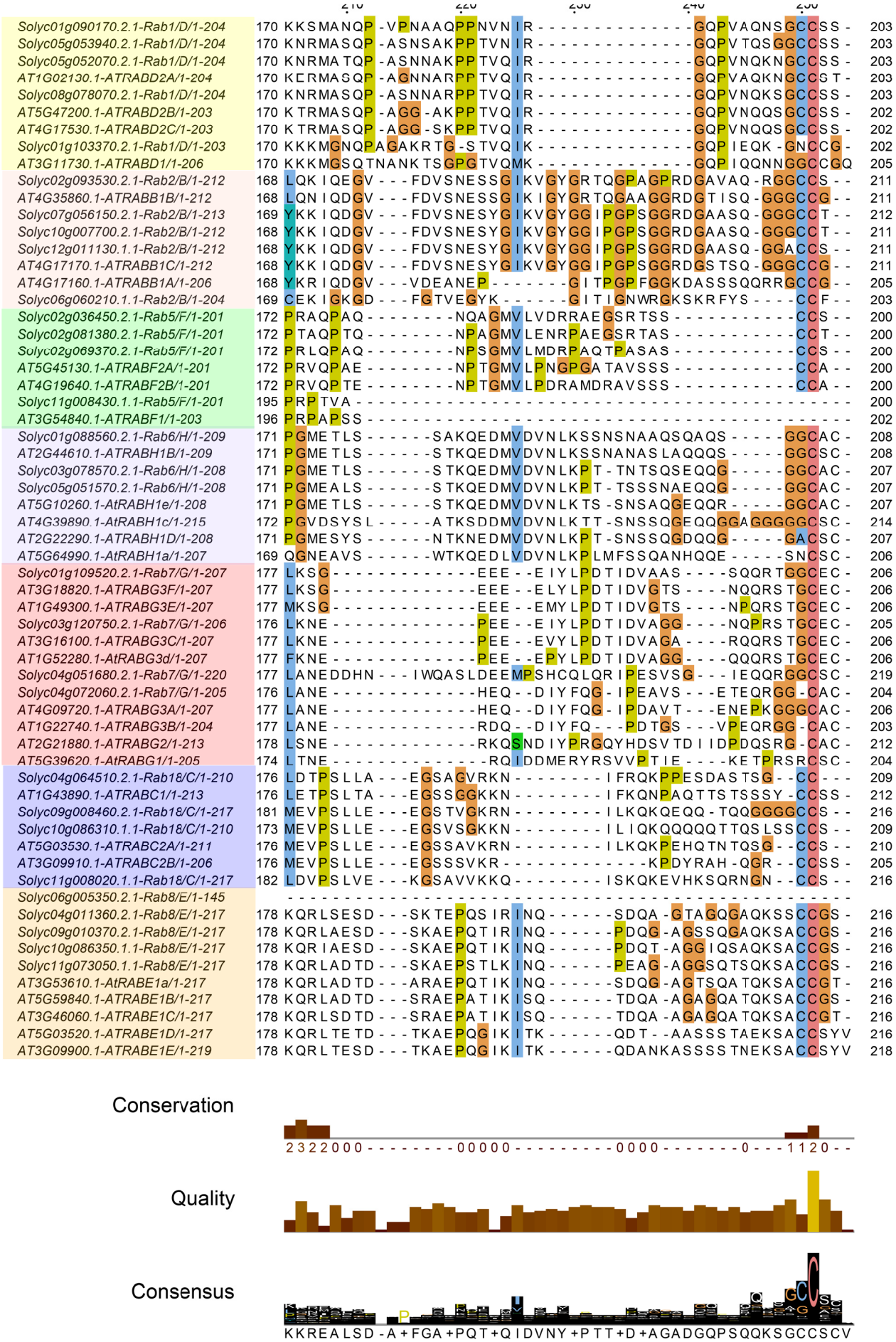


Fig.3.40 MSA of Arabidopsis and Tomato Rab1,2,5,6,7,8 and Rab18 GTPase cont...

A Neighbour joining phylogenetic tree by UPGMA method using 55 protein sequences of tomato Rab1, 2, 5, 6, 7, 8, 11 and Rab18 (Fig. 3.40.1) was generated using MEGA6 software using CLUSTAL alignment for generating multiple sequence alignment (MSA) for all tomato Rab GTPase (Fig.3.40.2). The individual tomato Rab GTPase falls in the same cluster of their respective groups. As shown in fig.3.40.1 the phylogenetic tree shows the following groups and clades

Clade1 consists of 25 tomato Rab11/RabA sequences. Rab 11/Rab A is further sub divided into A1, A2, A3, A4, A5 and A6.

- **Sub-Group1-RabA1** seven members (Solyc12g010790, Solyc07g053480, Solyc12g014080, Solyc03g006270, Solyc10g008840, Solyc02g072180 and Solyc07g055290)
- **Sub-Group2-RabA2** six members (Solyc01g103380, Solyc10g045550, Solyc03g079900, Soly05g052900, Solyc06g05810 and Solyc06g076450)
- **Sub-Group3-RabA3** with only one sequence (Solyc01g096220)
- **Sub-Group4-RabA4** with five members (Solyc04g012180, Solyc11g010100, Solyc01g086850, Solyc03g064020 and Solyc10g079030).
- **Sub-Group5-RabA5** five members (Solyc05g054150, Solyc11g012460, Solyc09g056340, Solyc09g097900 and Solyc09g098170).
- **Sub-Group6-RabA6** with only one sequence (Soly03g118820)

Clade2 consist of five members of tomato Rab2/RabB; (Solyc06g060210, Solyc07g056150, Solyc02g093530, Solyc10g007700 and Solyc12g011130)

Clade3 consists of four tomato Rab18/RabC GTPase; (Solyc09g008460, Solyc10g086310, Solyc11g008020 and Solyc04g064510)

Clade4 consists of five tomato Rab1/RabD members (Solyc05g053940, Solyc01g090170, Solyc08g078070, Solyc05g052070 and Solyc01g103370)

Clade5 consist of five tomato Rab8/RabE; (Solyc06g005350, Solyc10g086350, Solyc11g073050, Solyc04g011360 and Solyc09g010370)

Clade6 consist of four tomato Rab5/RabF; (Solyc11g008430, Solyc02g069370, Solyc02g036450 and Solyc02g081380)

Clade7 consists of three Rab6/RabH GTPase Solyc03g078570, Solyc05g051570 and Solyc01g088560).

Clade8 consist of four tomato Rab7/RabG GTPase (Solyc04g051680, Solyc04g072060, Solyc01g109520 and Solyc03g120750).

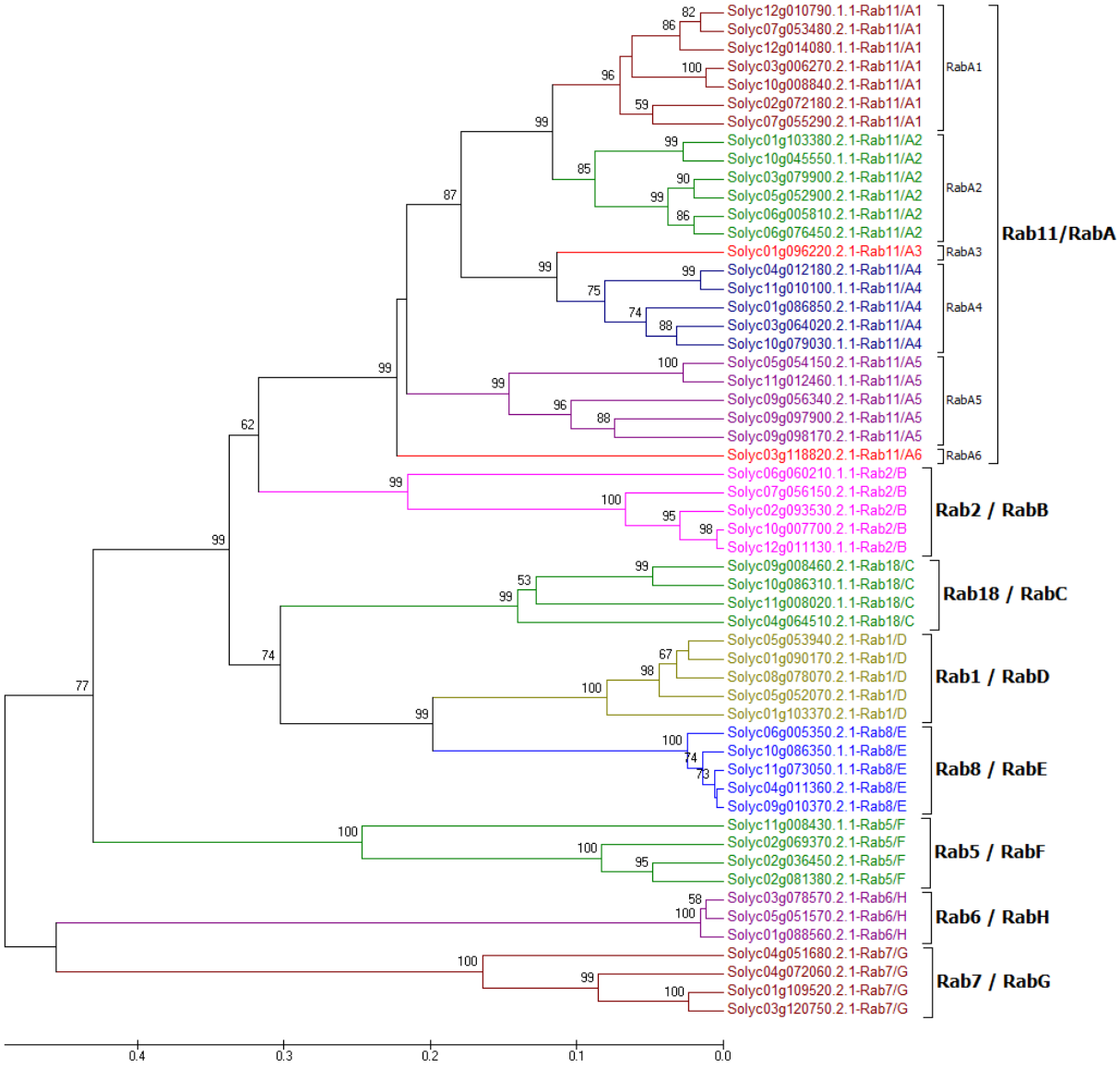


Fig. 3.40.1 Phylogenetic tree of all Tomato Rab GTPases Rab1, 2, 5, 6, 7, 8, 11 and Rab 18 (Solyc Ids-ITAG2.4). The evolutionary history was inferred using the UPGMA method. The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The evolutionary distances were computed using the Poisson correction method and are in the units of the number of amino acid substitutions per site. The analysis involved 55 amino acid sequences from Tomato ITAG2.4. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA6.

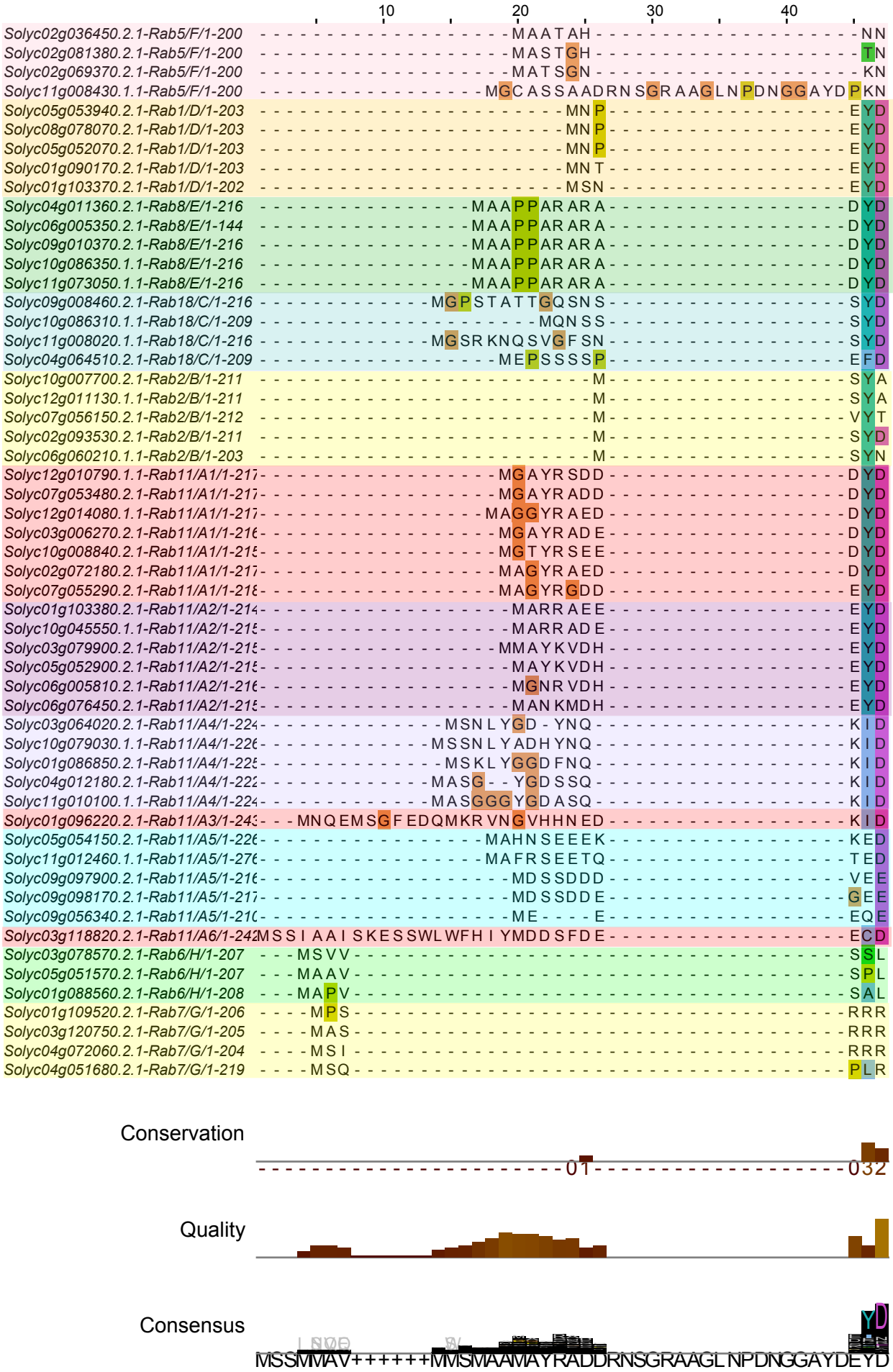


Fig.3.40.2 MSA of Tomato Rab1,2,5,6,7,8,11 and Rab18 GTPase with Solyc Ids ver. ITAG2.4 generated by Jalview version 2.8.2 showing the group conservation plot, histogram, and consensus logo.

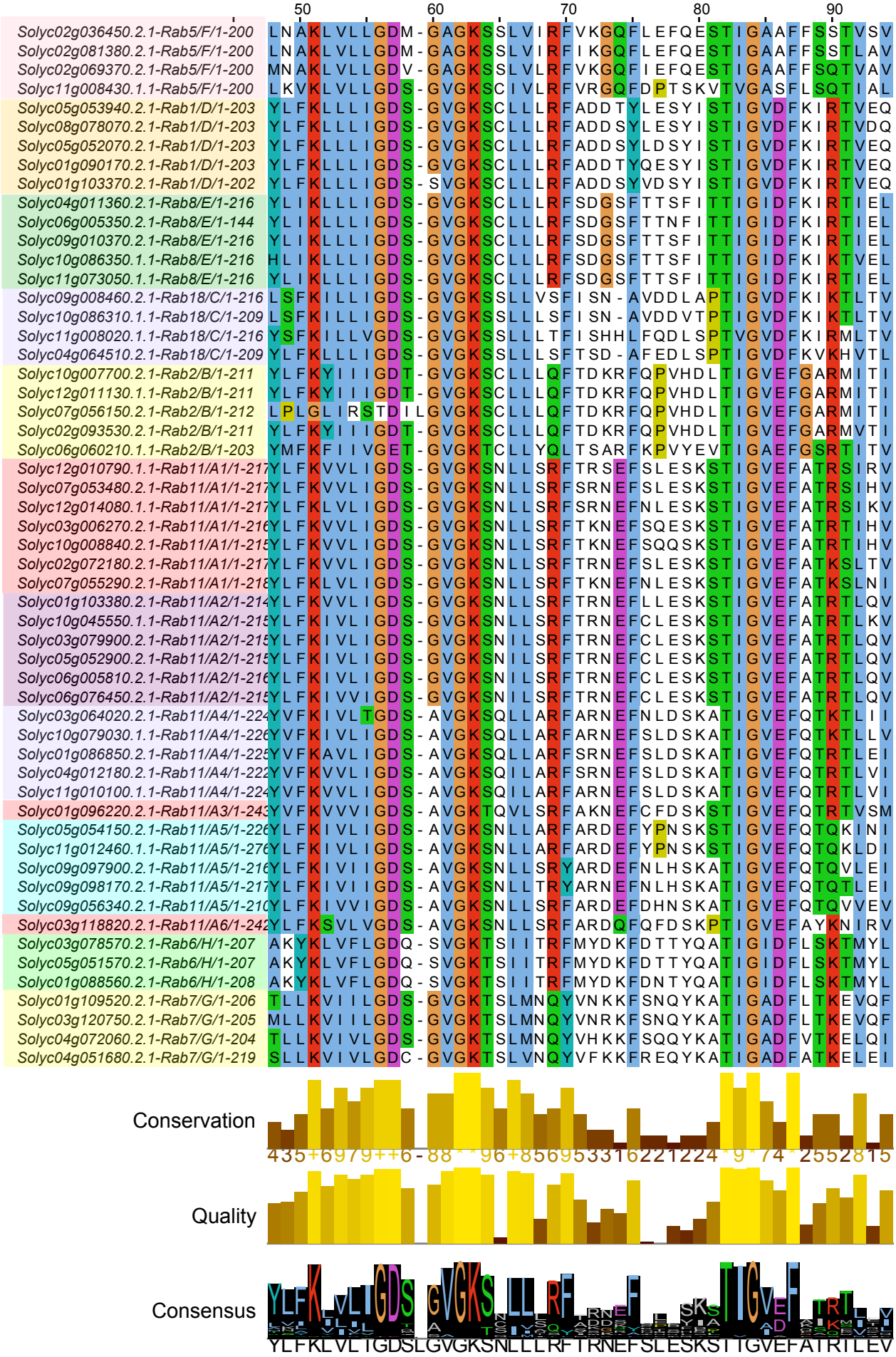


Fig.3.40.2 MSA of Tomato Rab1,2,5,6,7,8,11 and Rab18 GTPase with Solyc Ids ver. ITAG2.4 cont...

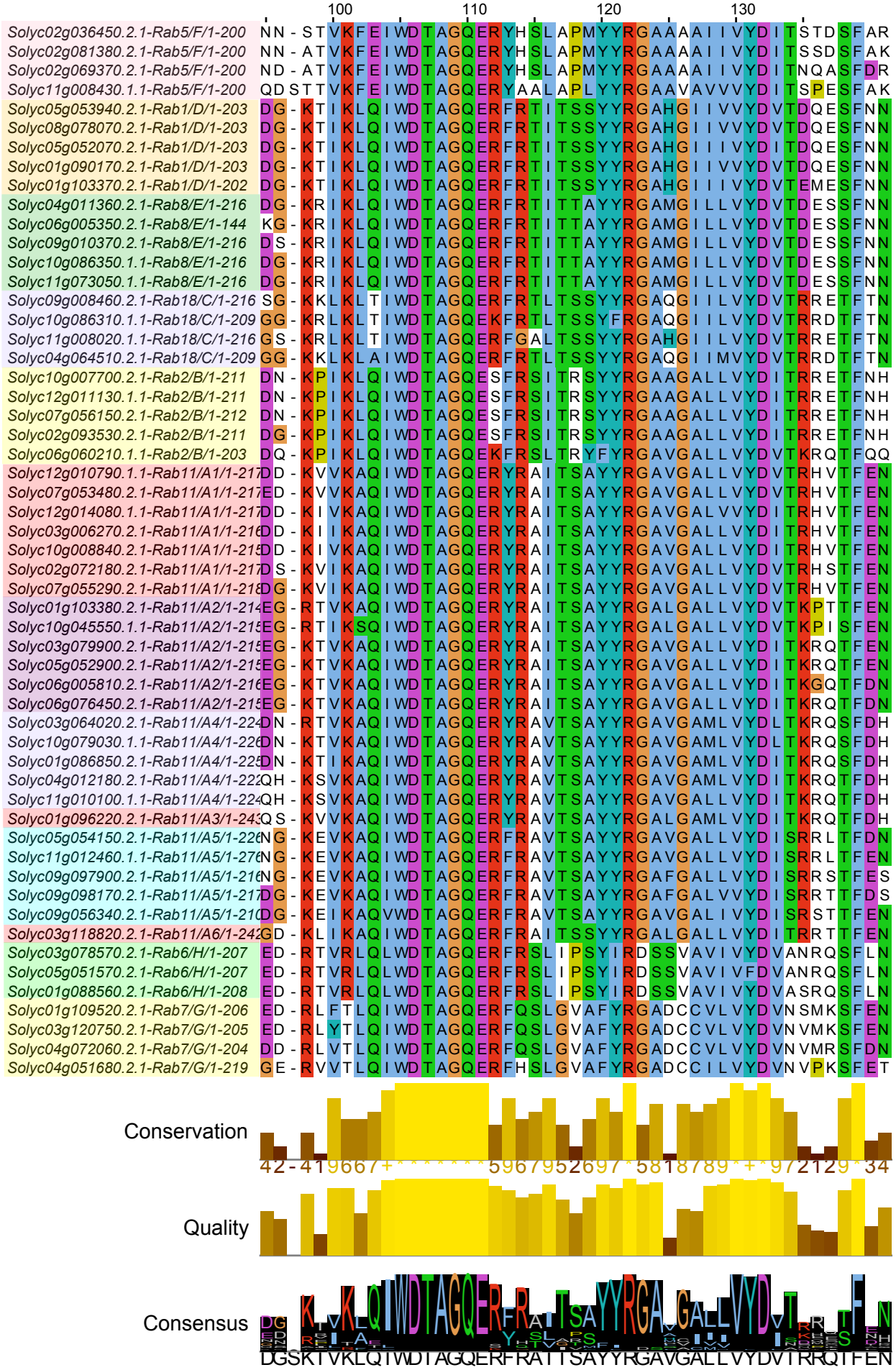


Fig.3.40.2 MSA of Tomato Rab1,2,5,6,7,8,11 and Rab18 GTPase with Solyc Ids ver. ITAG2.4 cont...

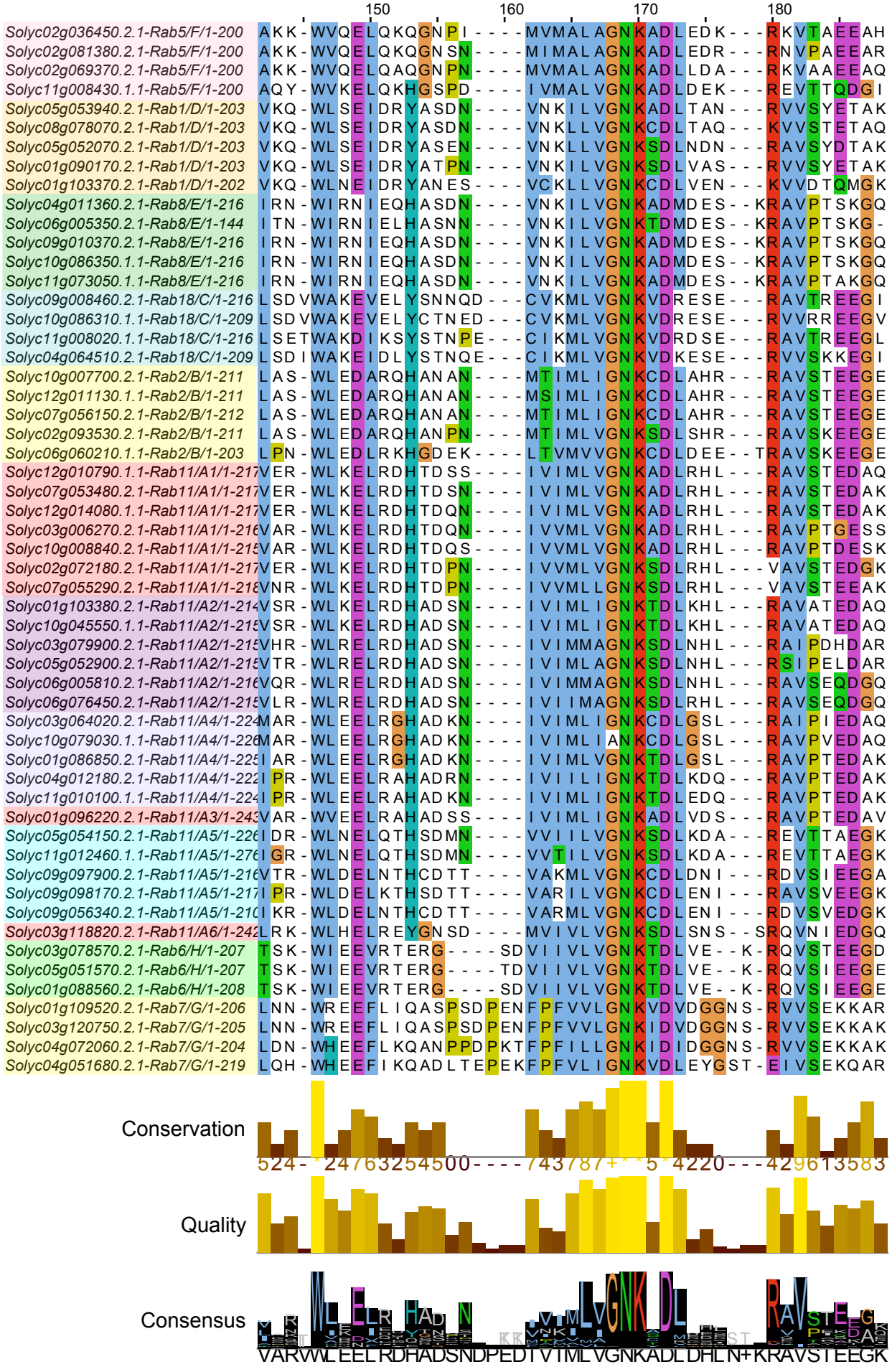


Fig.3.40.2 MSA of Tomato Rab1,2,5,6,7,8,11 and Rab18 GTPase with Solyc Ids ver. ITAG2.4 cont...

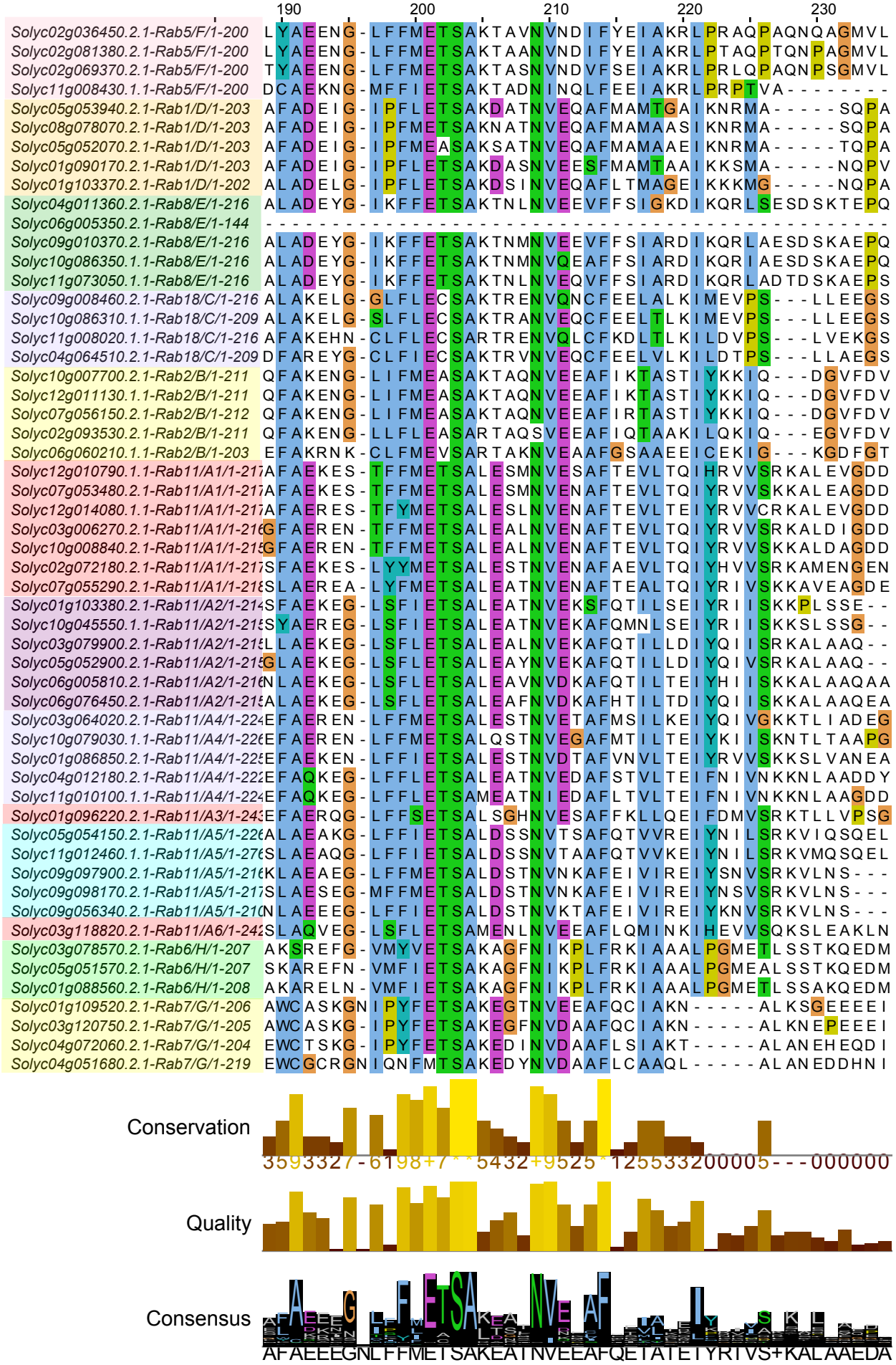


Fig.3.40.2 MSA of Tomato Rab1,2,5,6,7,8,11 and Rab18 GTPase with Solyc Ids ver. ITAG2.4 cont...

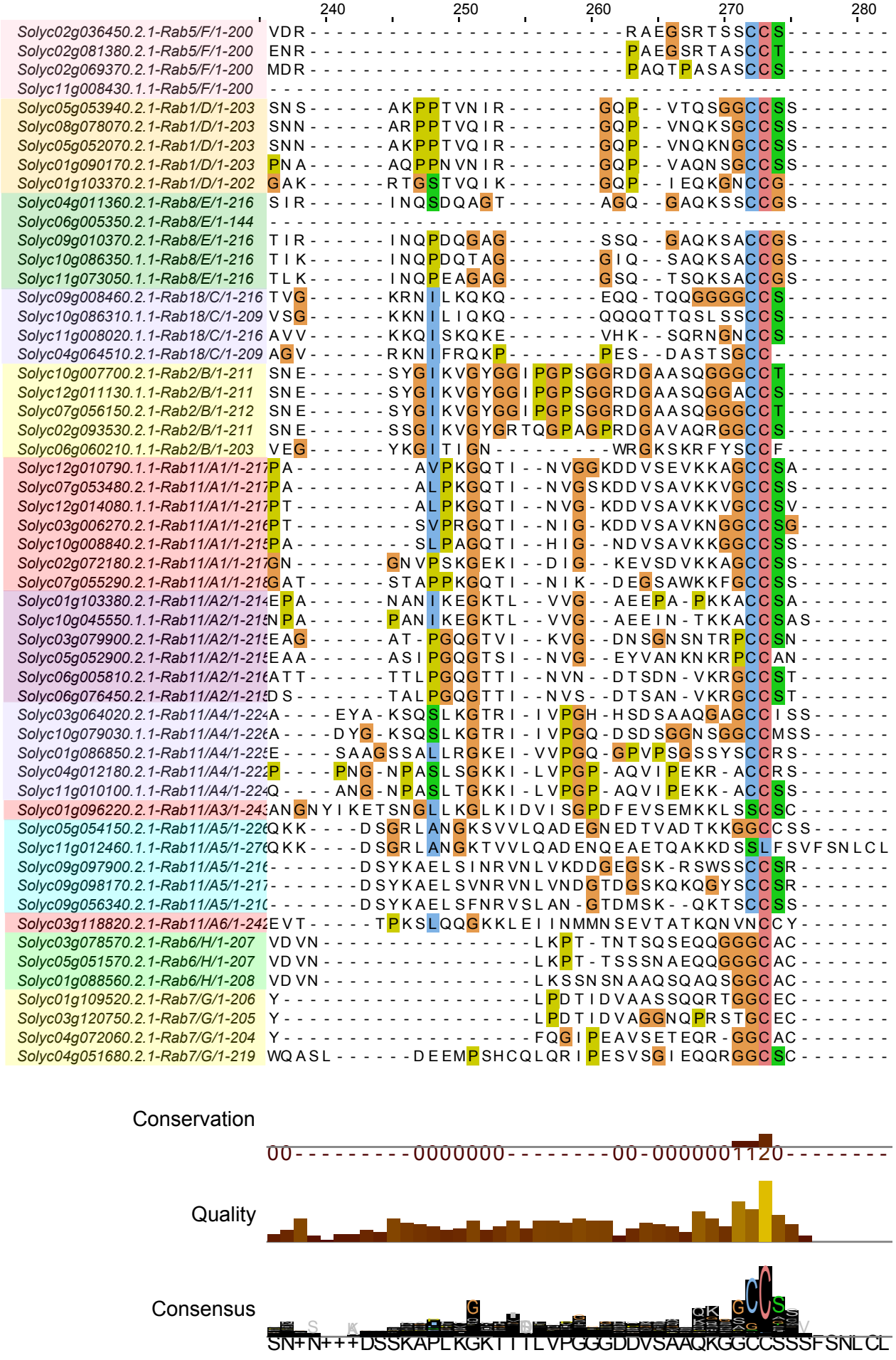


Fig.3.40.2 MSA of Tomato Rab1,2,5,6,7,8,11 and Rab18 GTPase with Solyc Ids ver. ITAG2.4 cont...

In order to validate the results tomato Rab GTPase protein sequences were aligned with Arabidopsis for MSA and phylogenetic tree was constructed to test the classification. The phylogenetic tree of Arabidopsis and Tomato Rab1, 2, 5, 6, 7, 8, 11 and Rab18 (Fig. 3.40.3) and the MSA (Fig.3.40.4) confirms the results and classification as discussed below

Clade1: Group I: - Rab11/RabA

Rab11/RabA is sub divided into six sub groups *viz.* A1, A2, A3, A4, A5 and A6.

RabA1: Clade1 consists a total of 16 RabA1 sub group with 7 tomato RabA1 GTPases falling in the same cluster with nine of Arabidopsis RabA1. Tomato RabA1a (Solyc07g055290) falls together with AtRabA1a.

RabA2: Clade2 consists of RabA2 sub group with six of tomato RabA2 falling in the same cluster with four of the Arabidopsis RabA2 GTPase. RabA2a of Arabidopsis has two members of tomato viz. Solyc01g103380 and Solyc10g045550.

RabA3: Clade 3 consists of RabA3 sub group with one member of tomato RabA3 (Solyc01g096220) falling in the same node with the Arabidopsis AtRabA3.

RabA4: Clade 4 consists of RabA4 sub group with five of tomato RabA4 falling in the same cluster with four of Arabidopsis RabA4.

RabA5: Clade5 consists of RabA5 sub group with five of tomato RabA5 falling in the same cluster with five of Arabidopsis RabA5. AtRabA5a has two members in tomato *viz.* Solyc05g054150 and Solyc11g012460.

RabA6: Clade 6 consists of RabA6 sub group with one member of tomato RabA6 (Soly03g118820) falling in the same cluster with the two Arabidopsis RabA6 *i.e* AtRabA6a and AtRabA6b.

Clade2: Group II- Five members of tomato Rab2/RabB GTPase (Solyc02g093530, Solyc06g060210, Solyc07g056150, Solyc10g007700 and Solyc12g011130) are clustered together in the same group with four of Arabidopsis Rab2 GTPase.

Clade3: Group III The four members of tomato Rab18/RabC (Solyc04g064510, Solyc09g008460, Solyc10g086310 and Solyc11g008020) fall in cluster with three Arabidopsis AtRab18/C. The sequence Solyc07g052410 was not included in the analysis.

Clade4: Group IV There are five members of tomato Rab1/RabD (Solyc01g090170, Solyc01g103370, Solyc05g052070, Solyc05g053940 and Solyc08g078070) falling in the same cluster with four AtRab1/RabD GTPase.

Clade5: Group V The five genes of tomato Rab8/RabE GTPase (Solyc04g011360, Solyc06g005350, Solyc09g010370, Solyc10g086350 and Solyc11g073050) fall together with 5 At Rab8/RabE.

Clade6 Group VI: Three members of Arabidopsis and four members of Tomato Rab5/RabE GTPase (Solyc02g036450, Solyc02g069370, Solyc02g081380 and Solyc11g008430) fall in the same cluster. Solyc12g005840 and Solyc05g045660 were not included in the present analysis.

Clade7: Group VII Five of AtRab6 fall together in the same cluster with three members of tomato Rab6/RabH (Solyc01g088560, Solyc03g078570 and Solyc05g051570).

Clade8: Group VIII Four members of tomato Rab7/RabG (Solyc04g051680, Solyc04g072060, Solyc01g109520 and Solyc03g120750) fall in the same cluster with seven members of AtRab7/RabG GTPase. The above results confirm and validate the classification and grouping of the current tomato Rab GTPases.

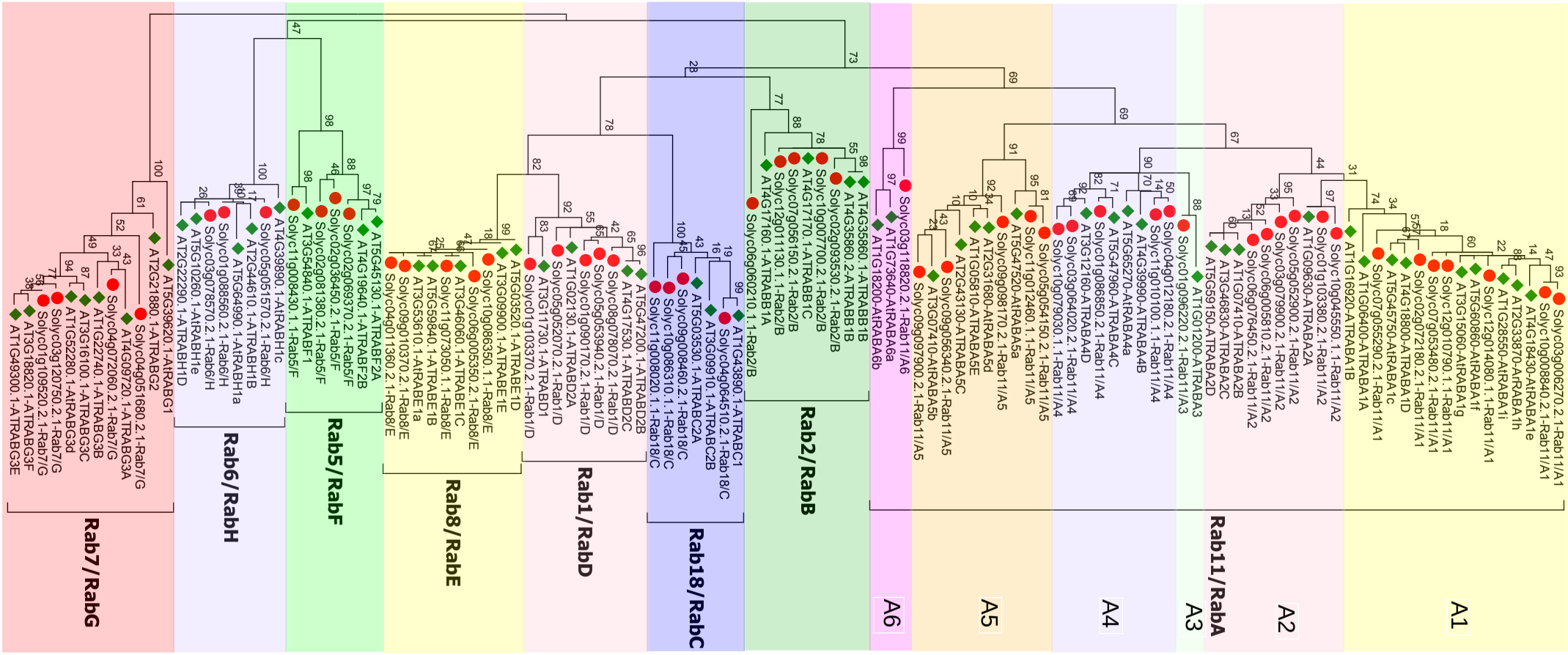


Fig.3.40.3 Molecular Phylogenetic tree of Arabidopsis (At) and Tomato (Solyc) Rab GTPases by Maximum Likelihood method based on protein sequences

The evolutionary history was inferred by using the Maximum Likelihood method based on the JTT matrix-based model. The tree with the highest log likelihood (-5003.3428) is shown. The percentage of trees in which the associated taxa clustered together is shown above the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 112 amino acid sequences 55 from Tomato and 57 from Arabidopsis. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA6. Individual Rab GTPase groups are highlighted. The red node marker represents Tomato-Solyc Ids (ITAG2.4) and the green diamond represents *Arabidopsis thaliana*- AT code (TAIR). Individual groups within Rab 11/Rab A class are shown as A1-A6 sub groups.

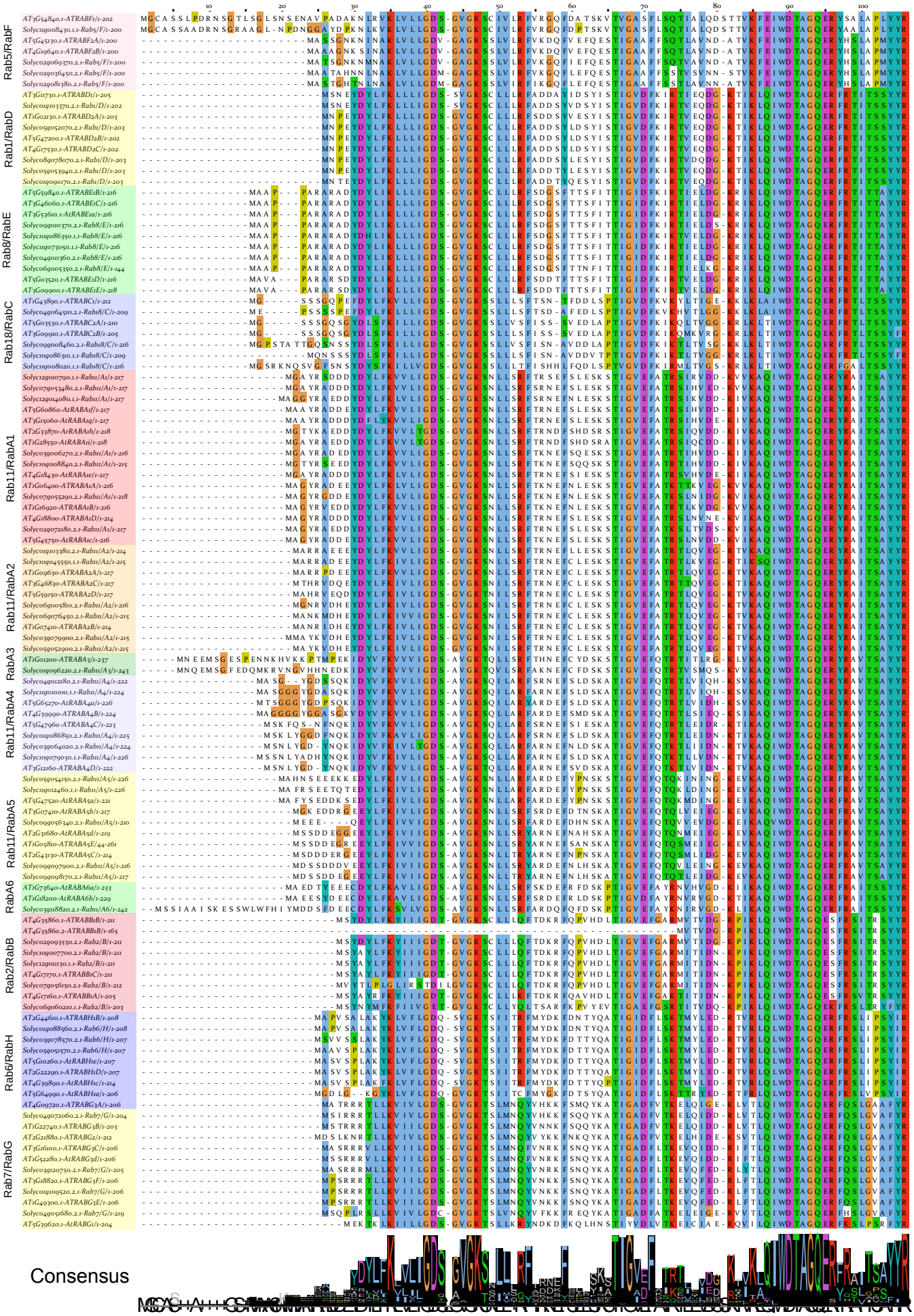


Fig.3.40.4 MSA of Arabidopsis and Tomato RAB GTPases including the group of Rab1,2,5,6,7,8,11 and Rab18 GTPase with tomato Solyc Ids ver. ITAG2.4 and Arabidopsis AGI codes. MSA was generated by Jalview version 2.8.2 showing the consensus logo. The individual groups are highlighted.

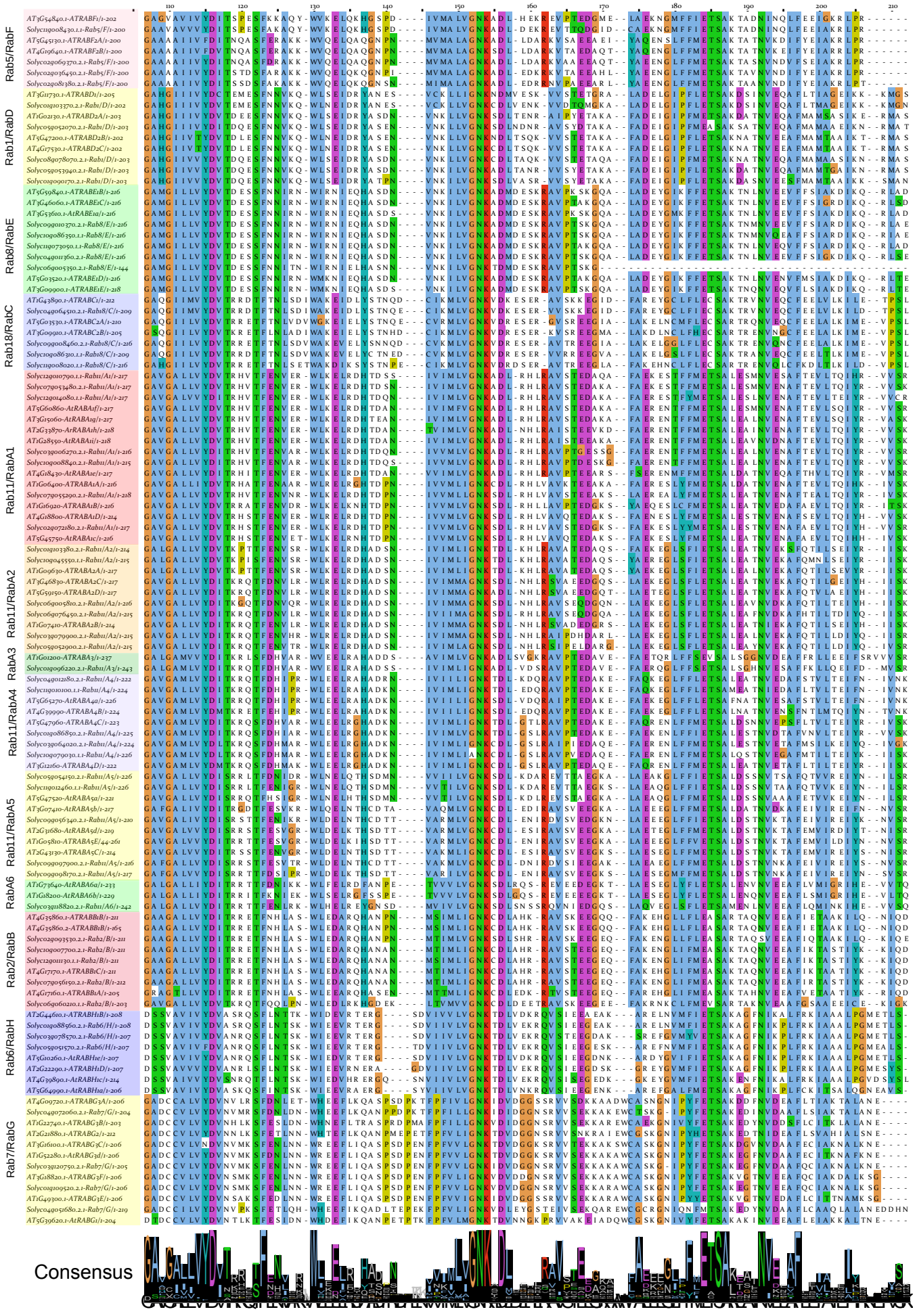


Fig.3.40.4 MSA of Arabidopsis and Tomato RAB GTPases including the group of Rab1,2,5,6,7,8,11 and Rab18 GTPase with tomato Solyc Ids ver. ITAG2.4 and Arabidopsis AGI codes Cont...

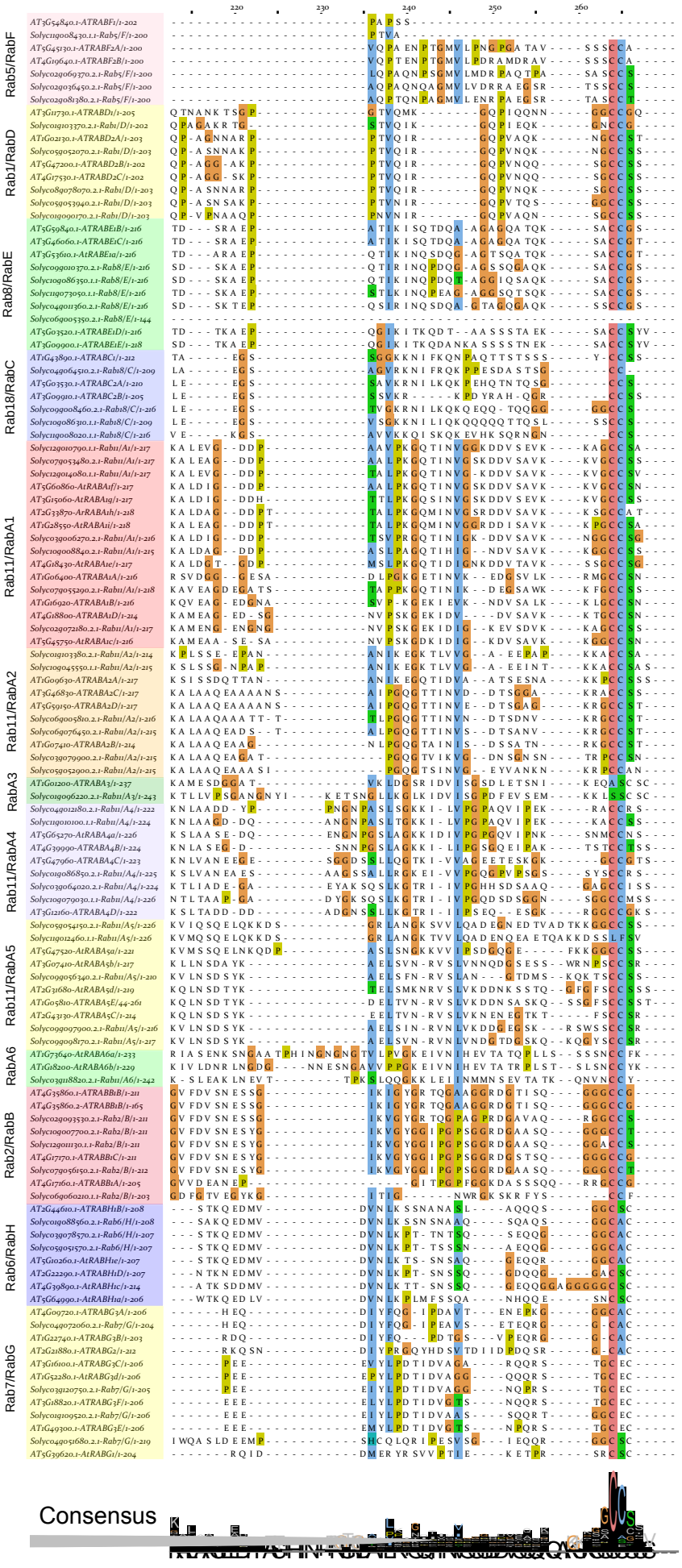


Fig.3.40.4 MSA of Arabidopsis and Tomato RAB GTPases including the group of Rab1,2,5,6,7,8,11 and Rab18 GTPase with tomato Solyc Ids ver. ITAG2.4 and Arabidopsis AGI codes Cont...

3.14 Solyc RAN GTPase

In case of Ran GTPase the BLAST hits and multiple sequence as well as domain architecture show that there are four putative Ran GTPases in tomato as compared to the four present in Arabidopsis. The results also indicate that for Arabidopsis AtRan4 (At5G55080) there are three orthologs in tomato *viz.,* Solyc01g104680, Solyc01g104700, as shown in the phylogenetic tree and domain analysis fig 3.41. The protein domain and alignment analyzer results from SGN also clearly highlight three tomato orthologs for AtRan4.The MSA of Arabidopsis and tomato RAN GTPase is shown in fig. 3.42.

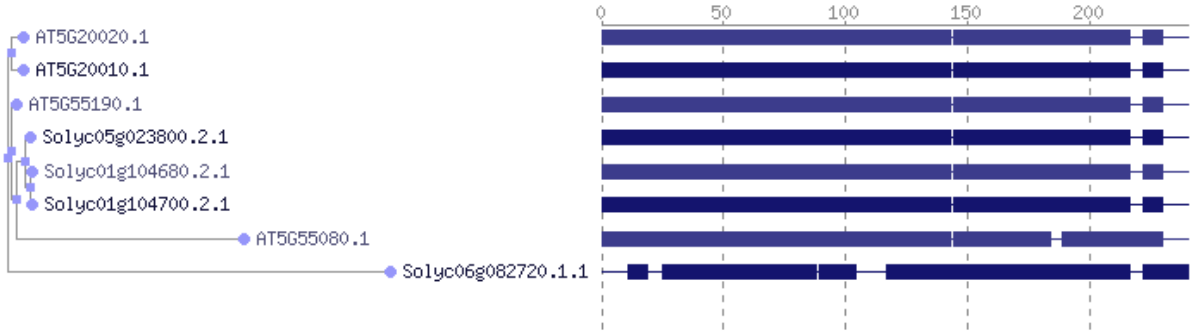


Fig3.41 Solyc Ran GTPase domain architecture and phylogenetic tree (SGN-ITAG2.4)

Orthologous pairs

AT5G55080.1: Solyc01g104680.2.1, Solyc01g104700.2.1, Solyc05g023800.
Solyc01g104680.2.1 AT5G55080.1
Solyc01g104700.2.1 AT5G55080.1
Solyc05g023800.2.1 AT5G55080.1
Solyc06g082720.1.1

One of the tomato sequence Solyc06g82720 annotated as one of the Ran GTPase falls as an outlier. Although the annotation of the sequence states it is Ran GTPase (Table 3.23) it is possible to be a Ran GTP binding protein (RanBP) which has a group of their own. Further analysis is required to find the other Ran related proteins, Ran Binding proteins and regulator of Ran GTPases.

3.15 Solyc ROP GTPase

The sequence analysis resulted in the identification of 10 ROP GTPase in tomato. Table 3.24 summarises the annotation, region, nucleotide sequences, mRNA and amino acid length of the respective tomato ROP GTPases. The BLAST results of Arabidopsis ROP GTPase into the SGN database to identify the Solyc ROP GTPase are shown in the supplemental files. The sequences were retrieved from database and were used for analysing the domain architecture of Arabidopsis and tomato ROP GTPase as shown in the fig. 3.43. The MSA of Arabidopsis and Tomato ROP GTPase is shown in fig.3.44. Most of tomato ROP GTPase confirm with the Arabidopsis ROP GTPase except one Solyc106055520.

Table 3.23 List of Tomato RAN GTPase Solyc Ids from SGN ITAG2.4 Release

Locus symbol	Chr	Annotation	Locus name	Region	Strand	NT	mRNA	Protein
Solyc01g104700	1	GTP-binding nuclear protein Ran-A1	Solyc01g104700.2	SL2.50ch01:93110761..93114261	-	3501	1,140	222
Solyc01g104680	1	GTP-binding nuclear protein Ran-A1	Solyc01g104680.2	SL2.50ch01:93099412..93103220	-	3,809	1,676	222
Solyc05g023800	5	GTP-binding nuclear protein Ran-A1	Solyc05g023800.2	SL2.50ch05:29353913..29357343	+	3,431	959	222
Solyc06g082720	6	GTP-binding nuclear protein Ran-A1	Solyc06g082720.1	SL2.50ch06:48442820..48443778	-	959	612	204
RAN GAP		**Ran GTPase activating Protein**						
Solyc01g079680	1	Ran GTPase activating protein Ran GAP2	Solyc01g079680.2	SL2.50ch01:78795201..78798172	+	2,972	2,077	551
Solyc09g065190	9	Ran GTPase activating protein Ran GAP1	Solyc09g065190.1	SL2.50ch09:63111331..63112935	-	1,605	1,605	535

Table 3.24 List of Tomato ROP GTPase Solyc Ids from SGN ITAG2.4 Release

Locus symbol	Chr	Annotation	Locus name	Region	Strand	NT	mRNA	Protein
Solyc01g088780	1	RhoC	Solyc01g088780.2	SL2.50ch01:83524888..83528574	+	3687	963	209
Solyc01g111560	1	RHO	Solyc01g111560.2	SL2.50ch01:97788642..97793788	+	5147	1,231	199
Solyc02g062020	2	Ras-like GTP-binding protein RHO	Solyc02g062020.2	SL2.50ch02:33605070..33610000	-	4931	1,236	198
Solyc02g077400	2	Rac-like GTP binding protein	Solyc02g077400.2	SL2.50ch02:42349603..42351610	-	2,008	697	198
Solyc02g083580	2	Ras-like GTP-binding protein RHO	Solyc02g083580.2	SL2.50ch02:46932950..46936218	-	3,269	1,319	198
Solyc03g033420	3	Rho-related GTP-binding protein RhoC	Solyc03g033420.2	SL2.50ch03:4981227..4985198	-	3,972	1,136	198
Solyc03g114070	3	Rac-like GTP-binding protein 4	Solyc03g114070.2	SL2.50ch03:64110442..64118342	-	7901	1,118	211
Solyc07g020800	7	Rac-like GTP-binding protein 4	Solyc07g020800.2	SL2.50ch07:13554611..13557326	-	2716	1,022	211
Solyc10g055520	10	GTP-binding RHO-like protein	Solyc10g055520.1	SL2.50ch10:56897420..56901268	+	3,849	480	160
Solyc12g007210	12	Ras-like GTP-binding protein RHO	Solyc12g007210.1	SL2.50ch12:1633954..1635822	-	1869	636	212

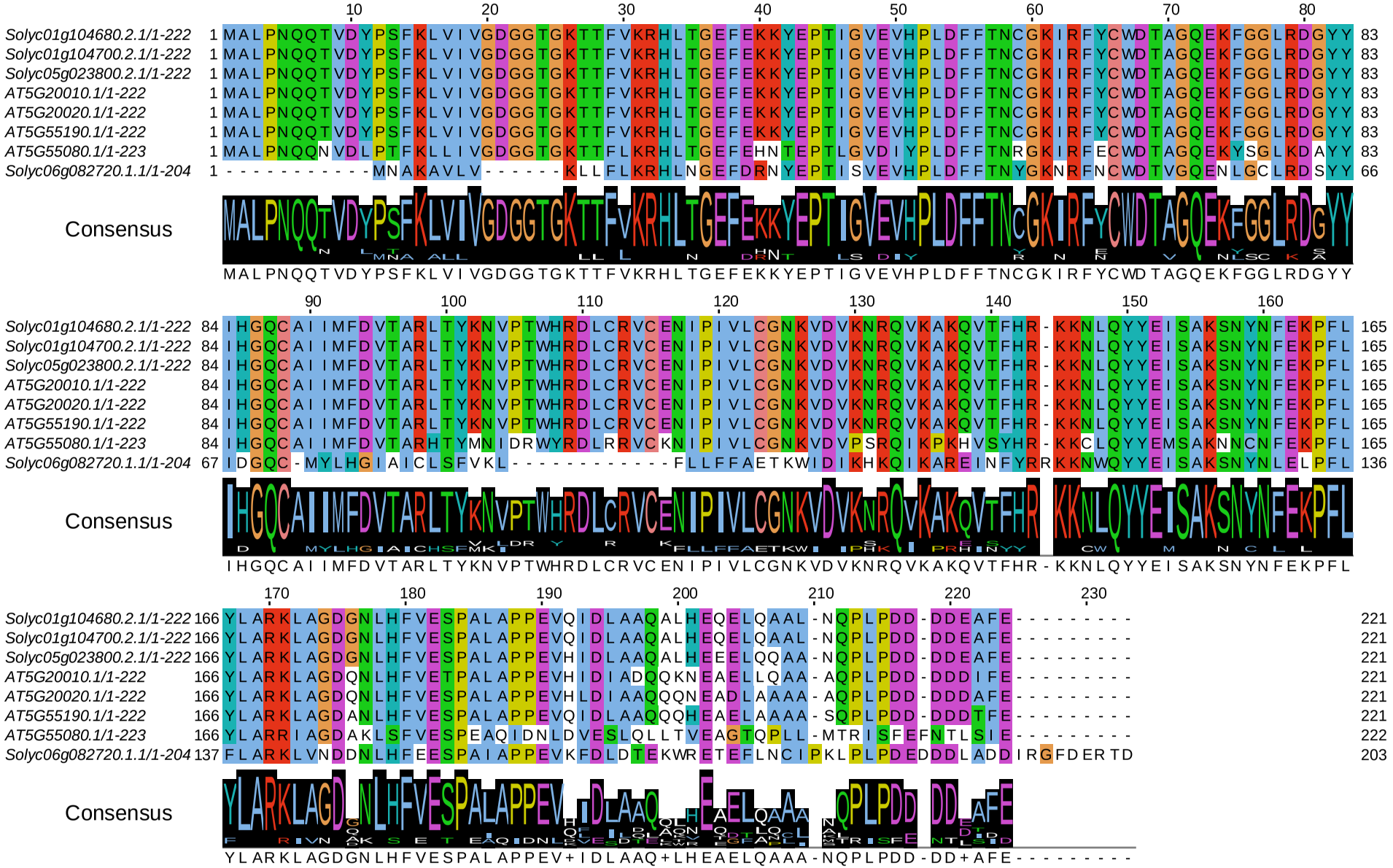


Fig.3.42 MSA of Arabidopsis and Tomato RAN GTPase (Solyc Id-ITAG2.4)

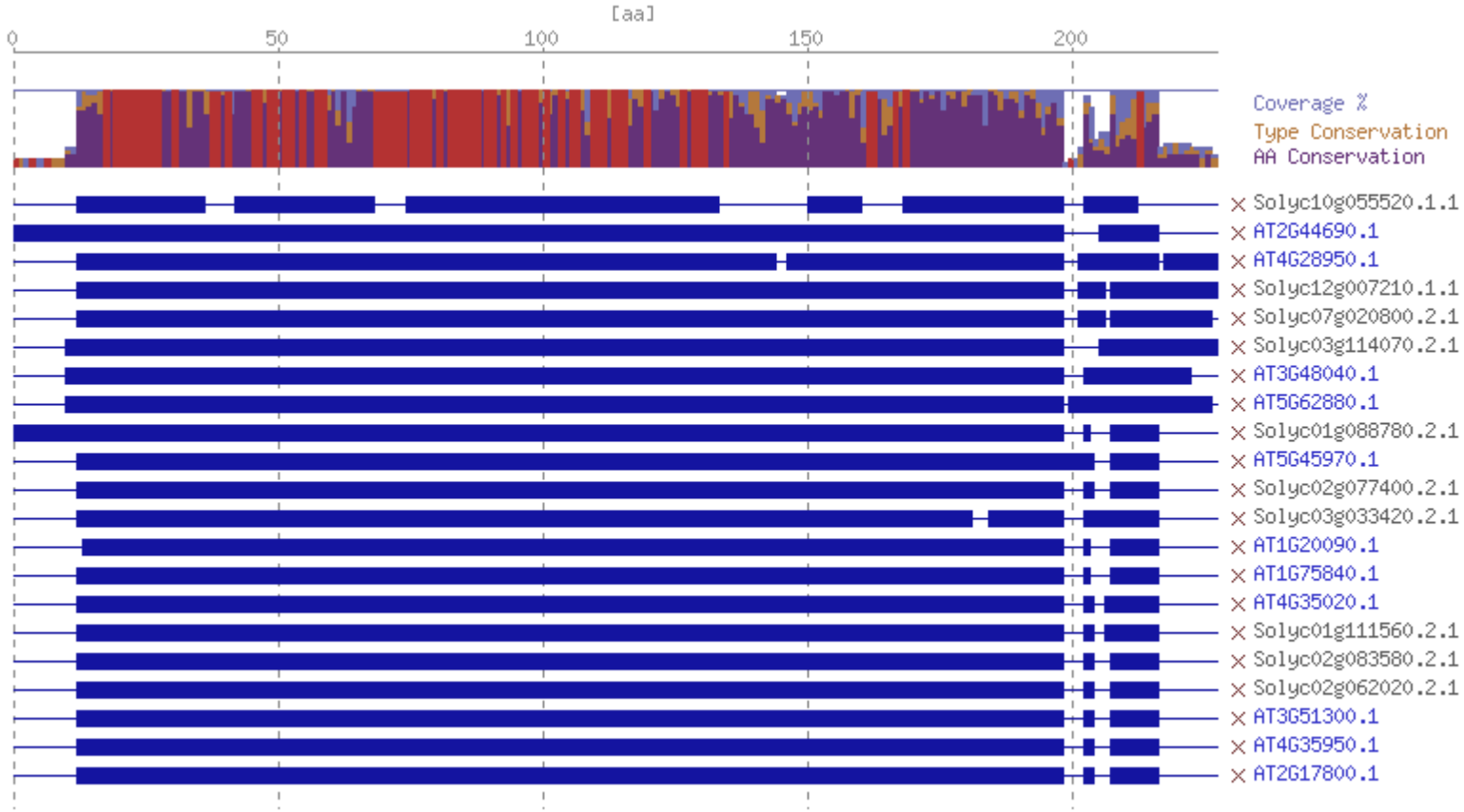


Fig. 3.43 Tomato and Arabidopsis ROP GTPase domain with Solyc Ids from SGN database (ITAG2.4)
The fig. above shows the domain architecture of Arabidopsis and tomato ROP GTPase aligned and grouped together. The Protein sequences were used to analyze the domain architecture in the SGN alignment analyzer. Fig. also shows the percentage of coverage, conservations plots and histogram for both.

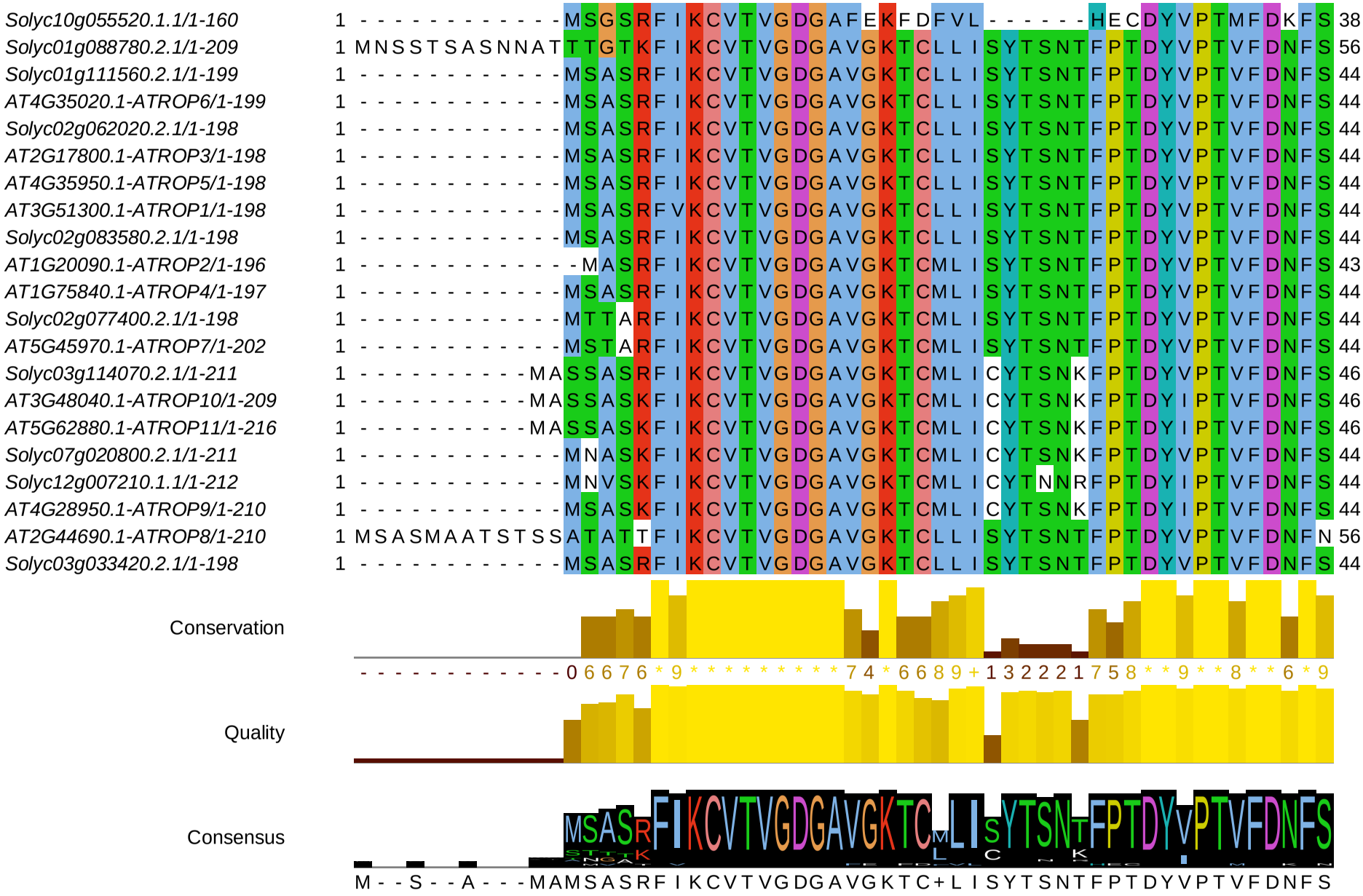


Fig.3.44 MSA of Arabidopsis and Tomato ROP GTPase

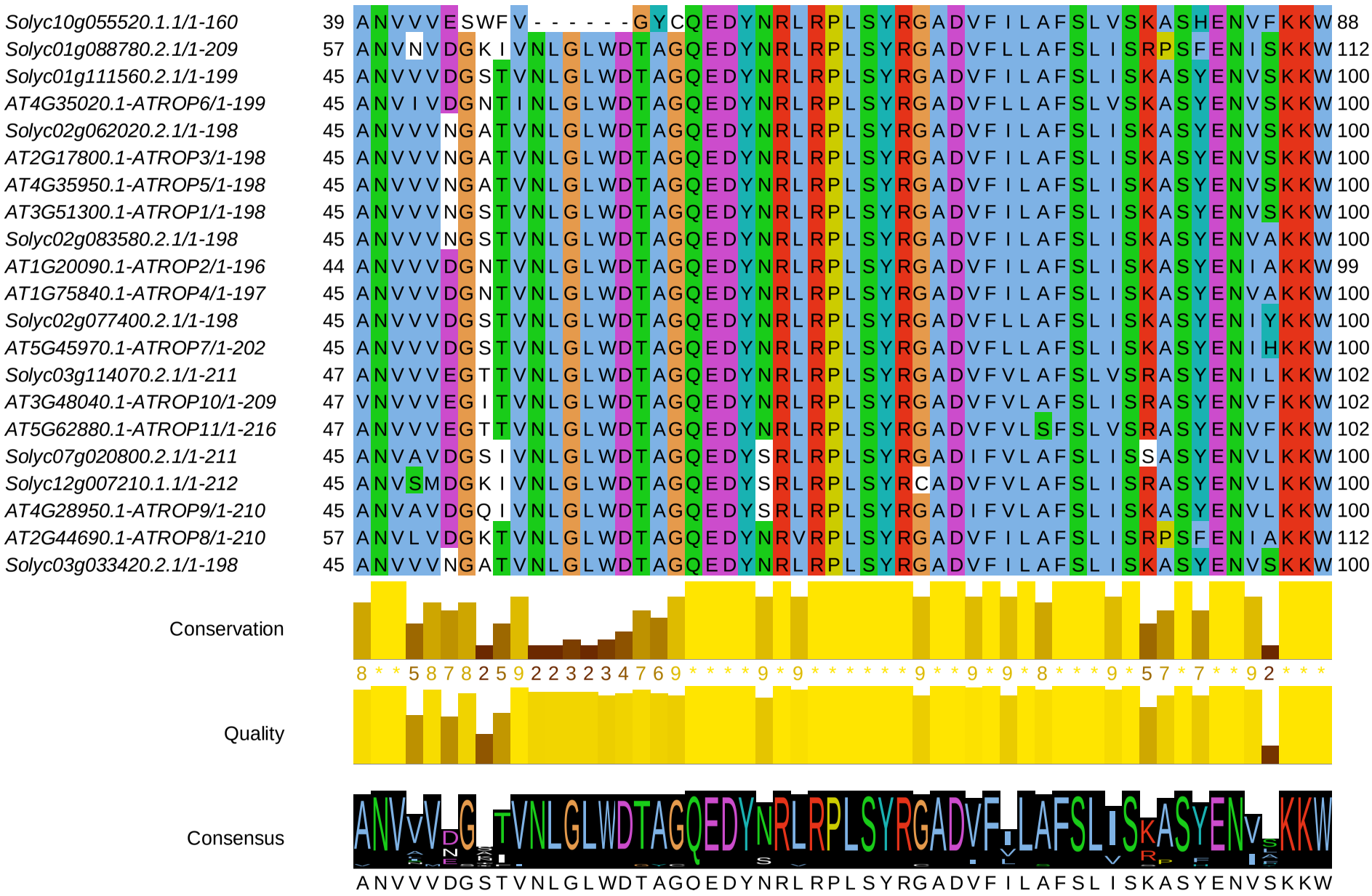


Fig.3.44 MSA of Arabidopsis and Tomato ROP GTPase cont...

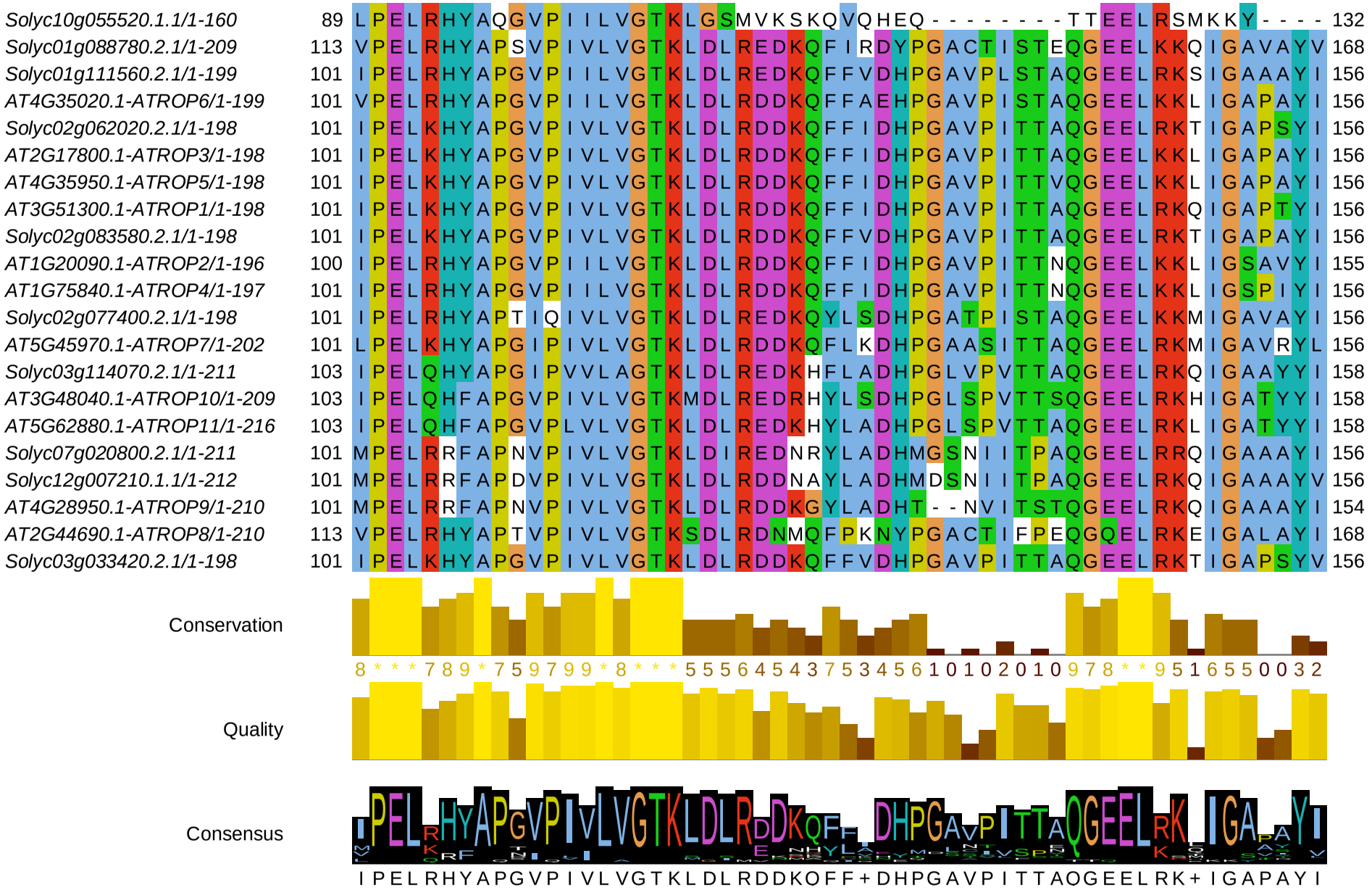


Fig.3.44 MSA of Arabidopsis and Tomato ROP GTPase cont...

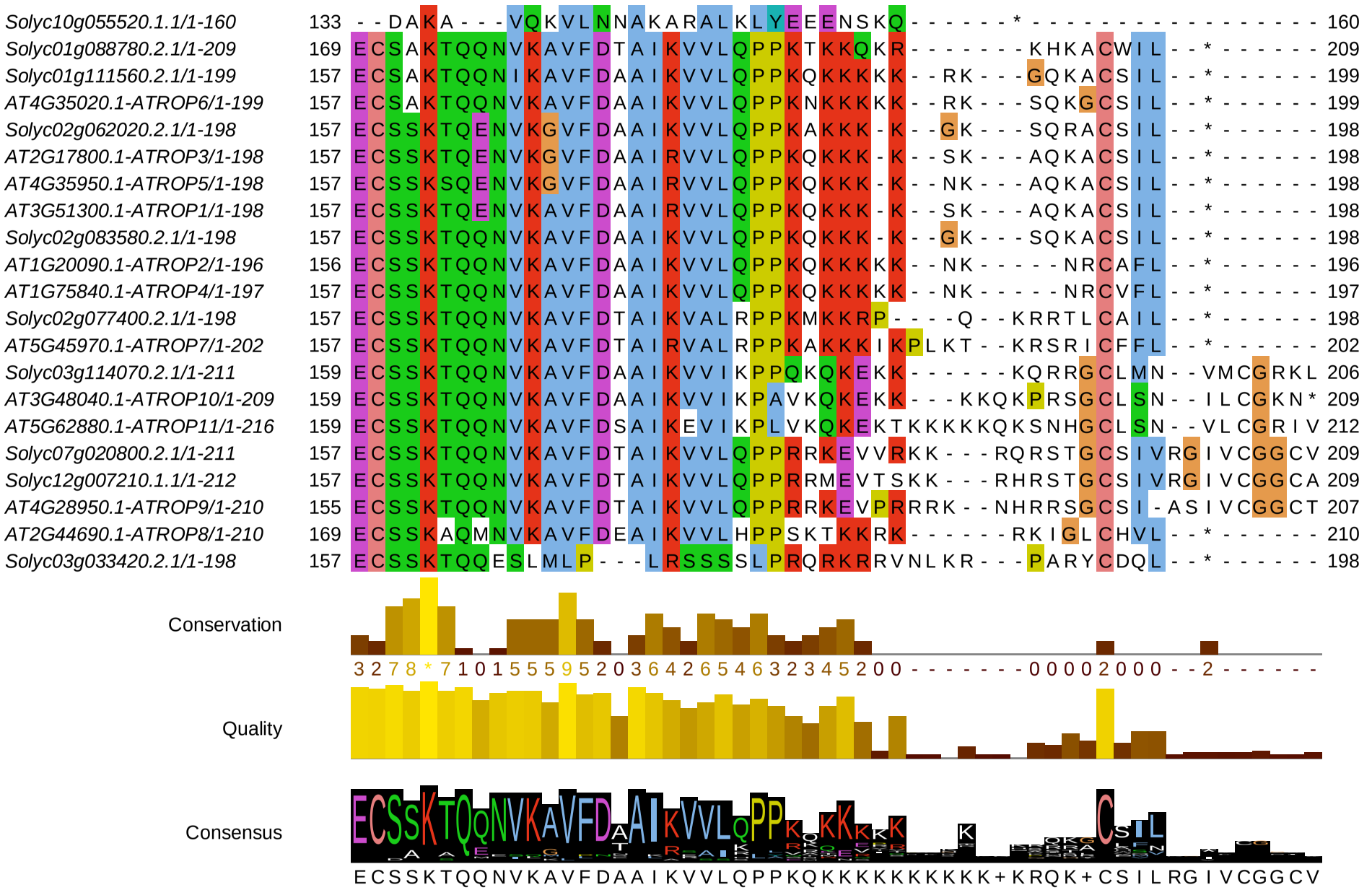


Fig.3.44 MSA of Arabidopsis and Tomato ROP GTPase cont...

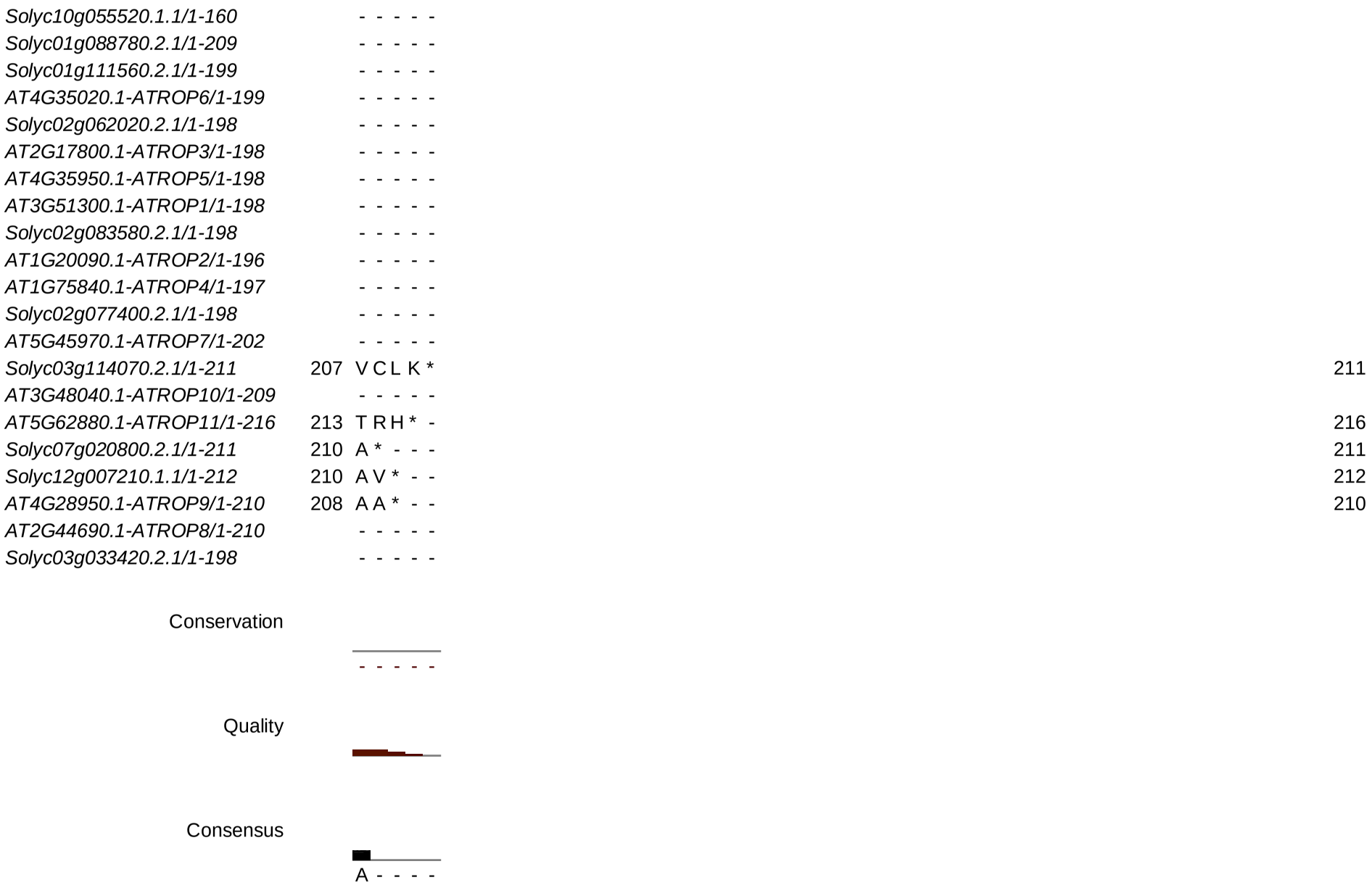


Fig.3.44 MSA of Arabidopsis and Tomato ROP GTPase cont...

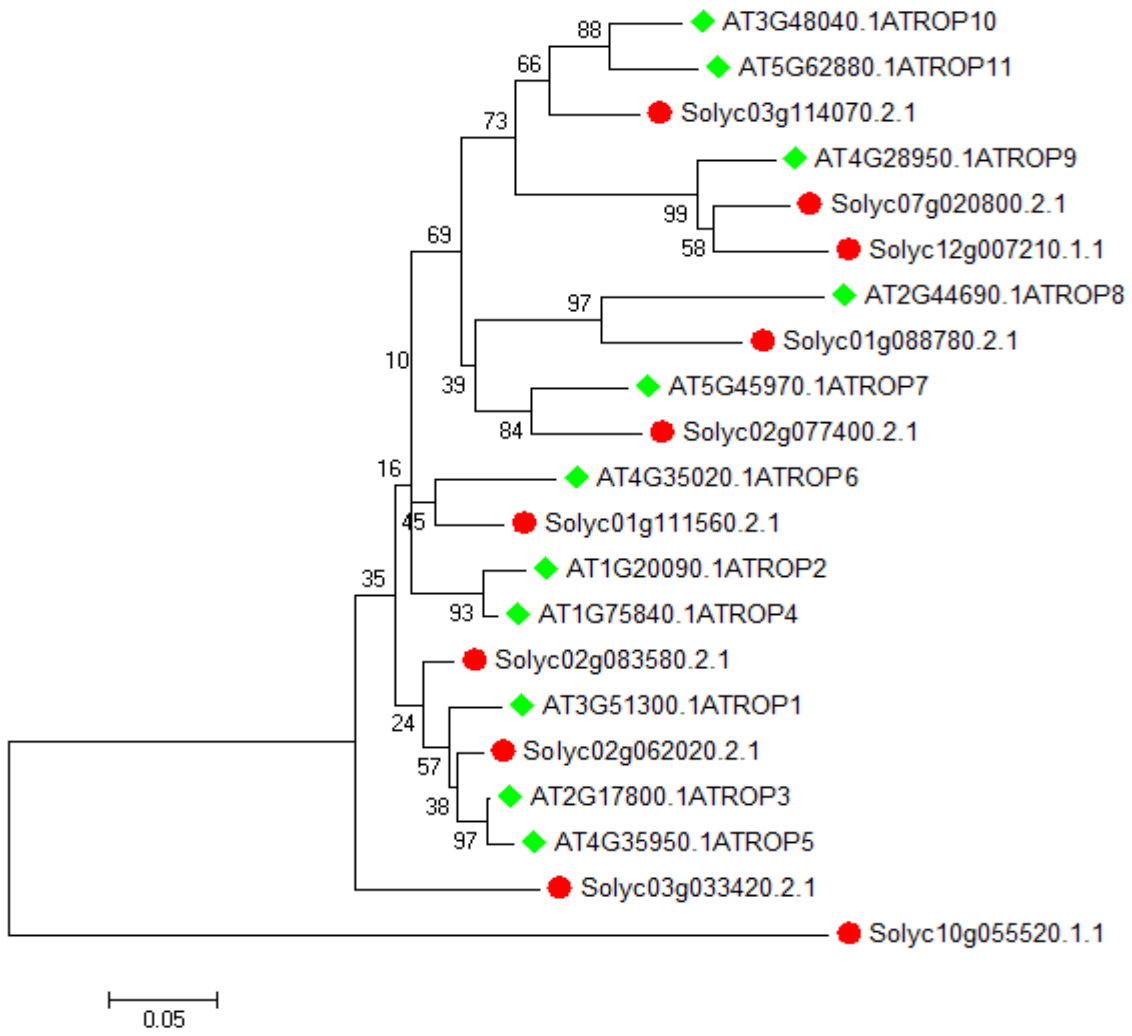


Fig. 3.45 Phylogenetic tree of At vs Solyc ROP GTPase

The phylogenetic tree of Arabidopsis (11) and Tomato Rop GTPases (10) was inferred using the Neighbor-Joining method using MEGA6. The optimal tree with the sum of branch length = 1.68 is shown. The percentages of replicate trees with the bootstrap test (1000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Poisson correction method and are in the units of the number of amino acid substitutions per site. The analysis involved 21 amino acid sequences. The node markers with red circle and green diamond show tomato and Arabidopsis sequence ids respectively

The phylogenetic tree of Solyc ROP GTPase and Arabidopsis (Fig. 3.45) revealed that Solyc03g114070 falls in the node with At ROP10 and AtROP11 GTPase. In case of AtROP9 there are two members of tomato ROPs *viz*., Solyc07g020800 and Solyc12g007210. Solyc01g088780 corresponds to the AtROP8 with clear single node likewise Solyc02g077400 and Solyc01g111560 fall in single node with At ROP7 and At ROP6 respectively. This confirms that theses tomato ROPs are the parallel members of ROP8, 7 and ROP6 with single node. Solyc02g083580 can be attributed to be a homolog of AtROP1. Solyc02g062020 falls in cluster with AtROP3 and AtROP5. Solyc10g055520 is an outlier in the group.

3.16 Solyc ARF GTPase

The other group of Small GTPase consists of ARF, ARL and SAR GTPases. Table 3.25 enlists tomato ARF GTPase with Solyc Ids from SGN (ITAG Release2.4). The files of BLAST results are in the supplemental folders. A total of 14 ARF GTPases were found in tomato, out of these only 12 were included in the present study. The sequences Solyc09g031870 and Solyc10g049260 were excluded from the analysis since they had short polypeptide of 50 and 60 aa respectively. In order to check the domain architecture of Tomato and Arabidopsis ARF GTPases the protein sequences were analyzed in SGN alignment analyzer (Fig. 3.46). The results indicate that the classification and grouping based on the alignment and domain clearly distinguishes the individual groups of ARF GTPases. Fig 3.48 Shows the MSA of the Solyc ARF and AtARF GTPases. Fig.3.47 shows the phylogenetic tree of Arabidopsis and tomato ARF GTPases. Clade 1 represents the group of AtARFA1 with six members of tomato ARFs clustered together with 7 members of putative tomato ARFA GTPase. The BLAST results show that Solyc04g025560 and Solyc12g099080 are more closely related and orthologous to AtARFA1E (AT3G62290). AtARF1B (AT5G14670) has the following putative orthologs in tomato *viz.* Solyc01g100870, Solyc09g055990 and Solyc10g049260. Solyc03g005300 falls in the node with AtARFC along with Solyc07g006640. In the group of ARFB Solyc09055990 lies close to AtARFB1A whereas, Solyc03g096910 and Solyc06g072800 fall together with AtARBB1B and AtARFB1C. Solyc03g096910 is an orthologous to AtARFB1B (AT5G17060).

3.17 Solyc SAR and ARL GTPase

Table 3.26 enlists Tomato SAR and ARL GTPase Solyc Ids from SGN ITAG2.4. In case of SAR GTPase four of Arabidopsis SAR GTPase fall in cluster with four tomato SAR GTPase viz. Solyc01g060130, Solyc01g079540, Solyc01g100350 and Solyc06g009660 (Fig3.51). The BLAST results indicated that there are six ARL GTPase in tomato (Table 3.26). A putative ARL Solyc00g020990 was not included in the study because of longer ORF (388aa) and Solyc00' code which means it is yet to be mapped to the respective chromosomes, but it had the IPR006689 for ARL GTPase. Fig. 3.49 shows the MSA and fig. 3.50 shows the domain architecture of Tomato and Arabidopsis SAR and ARL GTPase proteins. The phylogenetic tree of At and Solyc SAR and ARL GTPase (Fig. 3.51) classified the ARL into 3 groups ARLA, B and ARL C. Solyc07g014650 falls with AtARLC and Solyc10g074630 with ARLB. The other four tomato ARLs fall in the same cluster with AtARLA group likewise Solyc11g011040 falls in the same node with AtARLA1a.

Table 3.25 List of Tomato ARF GTPase Solyc Ids from SGN ITAG Release 2.4

Sr. No.	Locus symbol	Chr	Annotation	Locus name	Region	Strand	NT	mRNA	Protein
1	Solyc01g008000	1	ADP-ribosylation factor	Solyc01g008000.2	SL2.50ch01:2175122..2179207	+	4,086	891	210
2	Solyc01g100860	1	ADP-ribosylation factor	Solyc01g100860.2	SL2.50ch01:90764062..90773122	-	9,061	1,016	182
3	Solyc01g100870	1	ADP-ribosylation factor	Solyc01g100870.2	SL2.50ch01:90769525..90773125	-	3,601	1,088	211
4	Solyc02g092970	2	ADP ribosylation factor	Solyc02g092970.2	SL2.50ch02:53920842..53921843	+	1,002	592	192
5	Solyc03g005300	3	ADP-ribosylation factor	Solyc03g005300.2	SL2.50ch03:167876..173442	-	5,567	1,041	184
6	Solyc03g096910	3	ADP-ribosylation factor	Solyc03g096910.2	SL2.50ch03:59276338..59283003	-	6,666	592	133
7	Solyc04g025560	4	ADP-ribosylation factor	Solyc04g025560.2	SL2.50ch04:21891892..21896543	+	4,652	815	254
8	Solyc05g005190	5	ADP-ribosylation factor	Solyc05g005190.2	SL2.50ch05:170330..172998	+	2,669	734	182
9	Solyc06g072800	6	ADP-ribosylation factor	Solyc06g072800.2	SL2.50ch06:44895873..44901336	+	5,464	1,013	133
10	Solyc07g006640	7	ADP-ribosylation factor	Solyc07g006640.2	SL2.50ch07:1506417..1517122	+	10,706	905	183
11	Solyc09g055990	9	ADP-ribosylation factor	Solyc09g055990.1	SL2.50ch09:46622400..46627816	+	5,417	546	182
12	Solyc12g099080	12	ADP-ribosylation factor	Solyc12g099080.1	SL2.50ch12:66355679..66359958	-	4,280	672	224
13	Solyc09g031870	9	ADP-ribosylation factor	Solyc09g031870.2	SL2.50ch09:27678930..27680000	-	1,071	279	50
14	Solyc10g049260	10	ADP-ribosylation factor	Solyc10g049260.1	SL2.50ch10:44748784..44749402	+	619	180	60

The gene Solyc09g031870 and Solyc10g049260 highlighted with red font represent incomplete ORF and were not included in the present study.

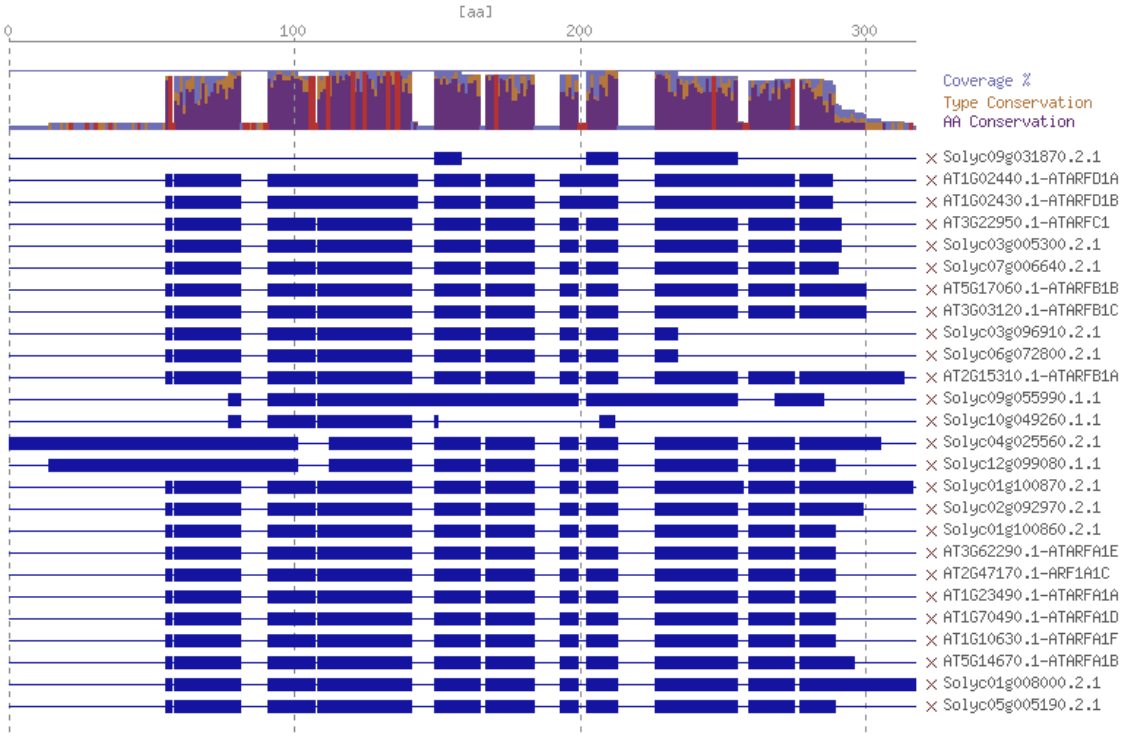


Fig. 3.46 Tomato and Arabidopsis ARF GTPase protein domain with Solyc Ids from SGN database (ITAG2.4). The fig. shows the domain architecture of Arabidopsis and tomato ARF GTPase aligned and grouped together. The Protein sequences were used to analyze the domain architecture in the SGN alignment analyzer. Fig. also shows the percentage of sequence coverage, conservations plots and histogram for both. Solyc09g031870 and Solyc10g049620 have very short polypeptide sequence.

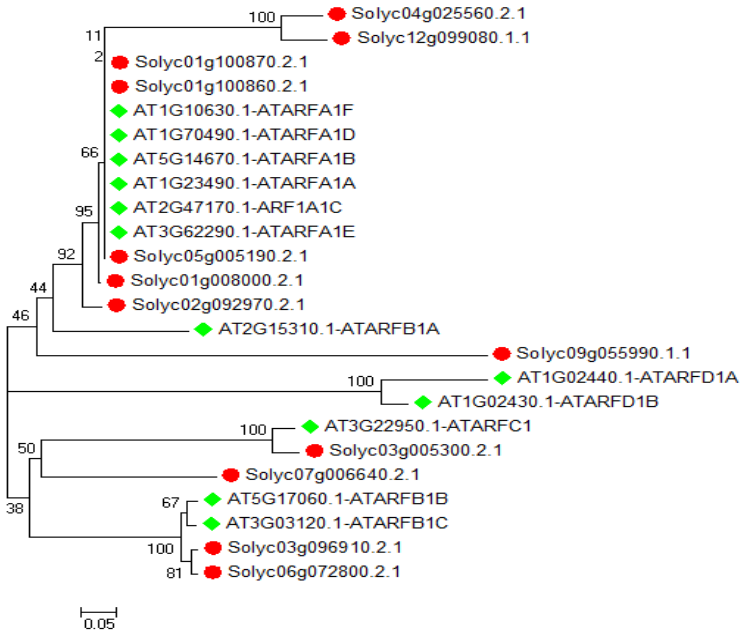


Fig. 3.47 Phylogenetic tree of Arabidopsis and Tomato ARF GTPase protein with Solyc Ids from SGN database (ITAG2.4). The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 3.97 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Poisson correction method and are in the units of the number of amino acid substitutions per site. The analysis involved 24 amino acid sequences with 12 sequences of Arabidopsis and Tomato each. The four ARFA, B, C and ARF D groups are visible with 7 ARF A, 3 ARFB and 2 ARFC in tomato. Evolutionary analyses were conducted in MEGA6.

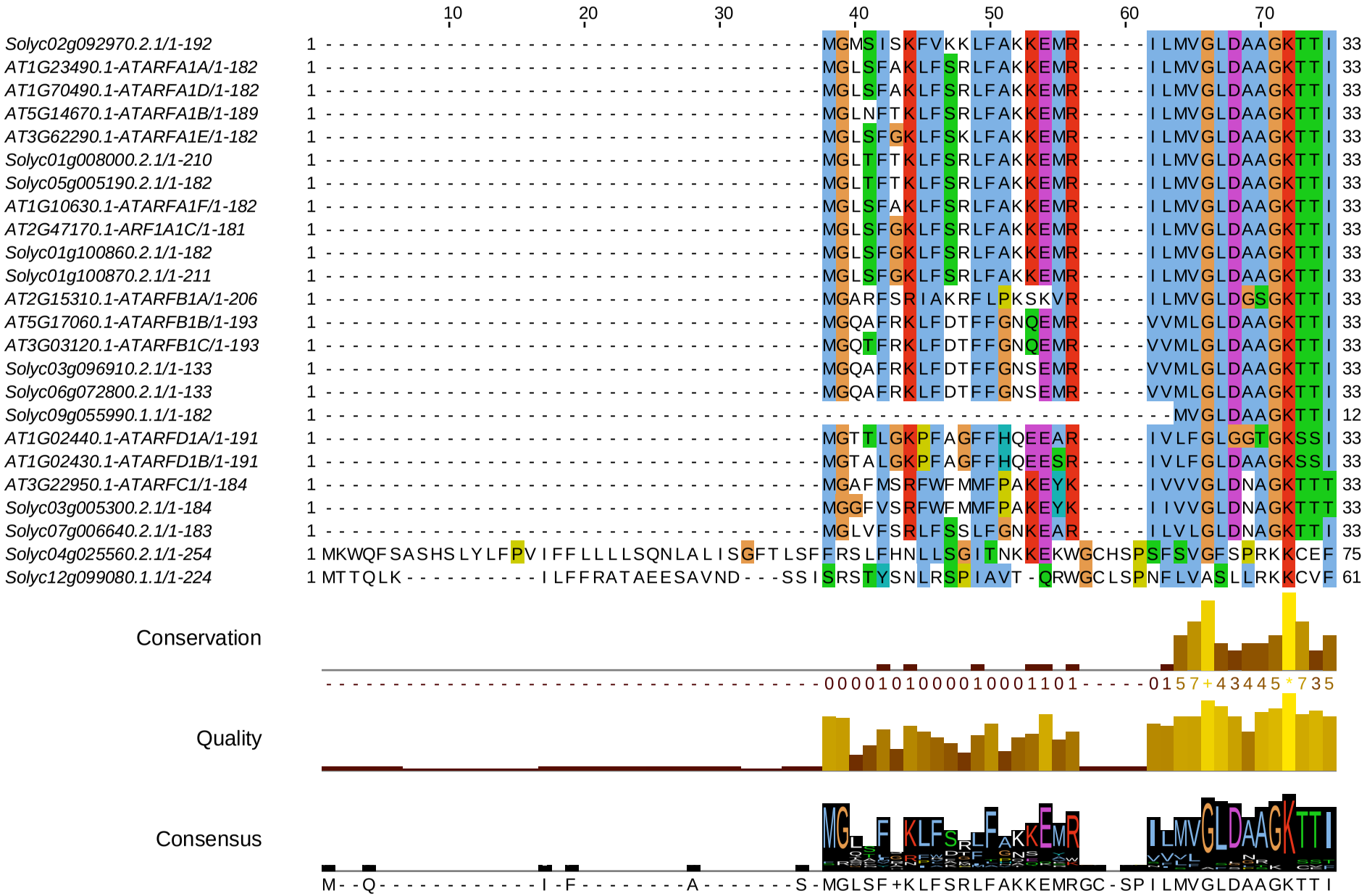


Fig.3.48 MSA of Arabidopsis and Tomato ARF GTPase

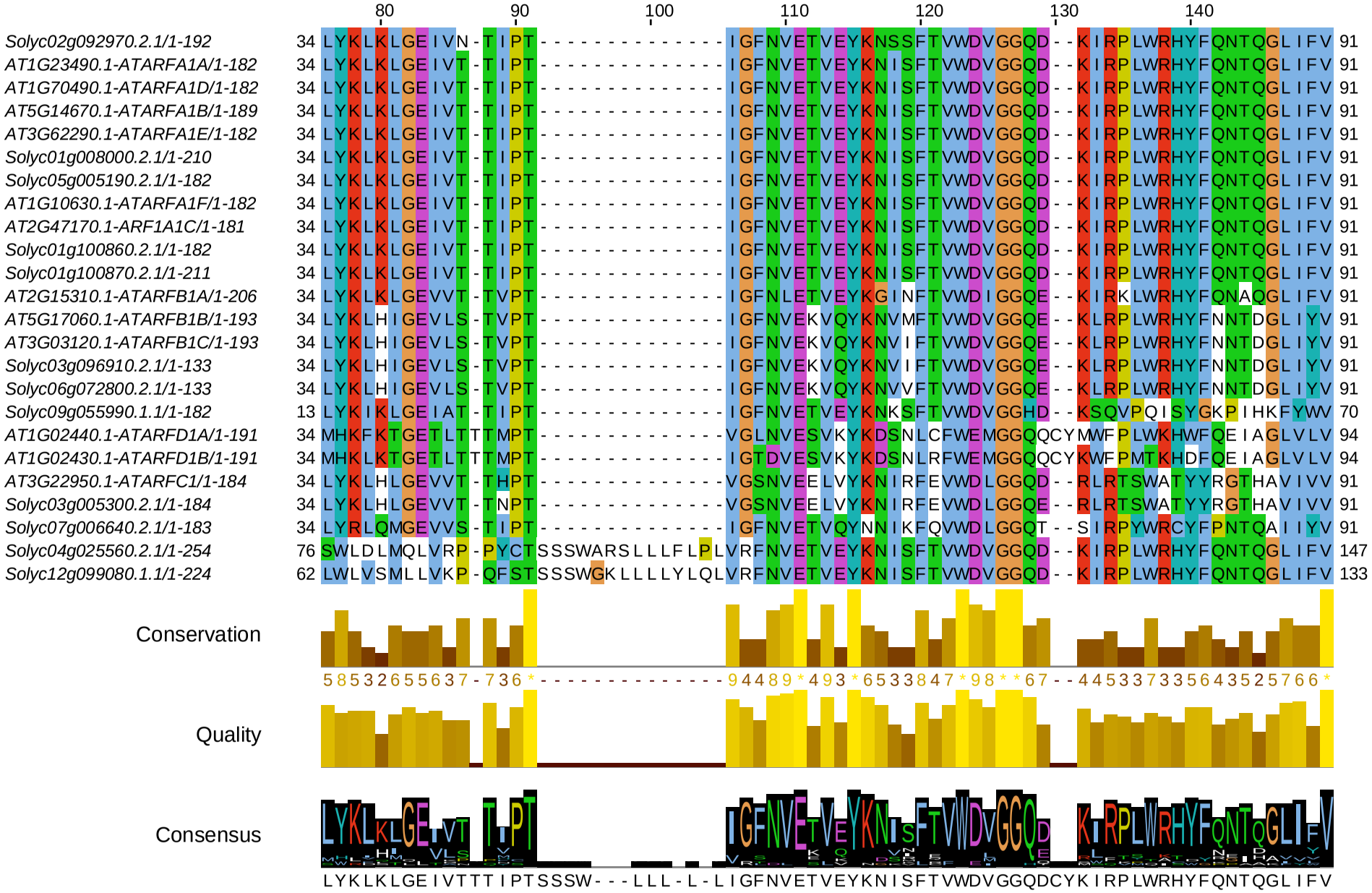


Fig.3.48 MSA of Arabidopsis and Tomato ARF GTPase cont...

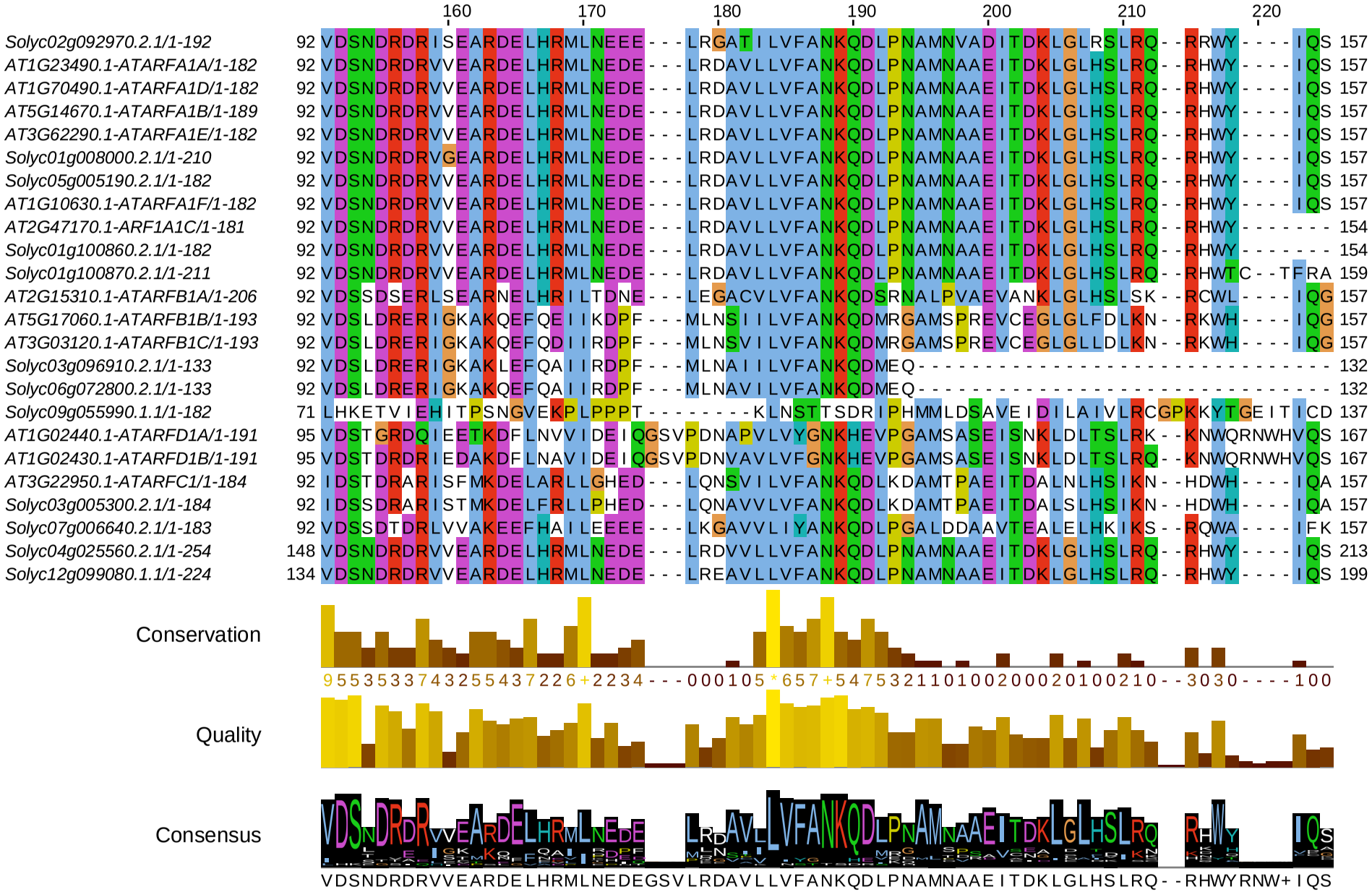


Fig.3.48 MSA of Arabidopsis and Tomato ARF GTPase cont...

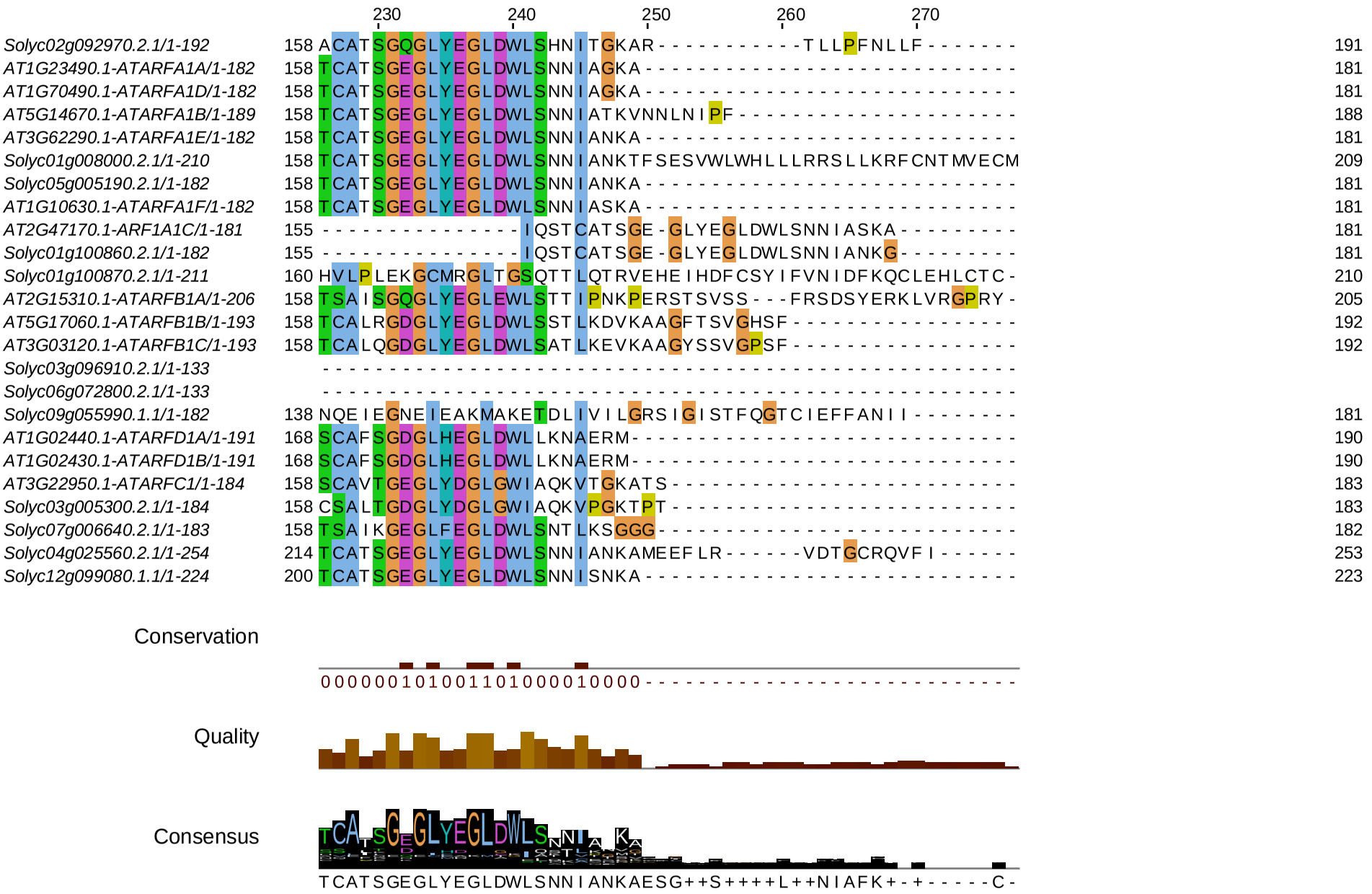


Fig.3.48 MSA of Arabidopsis and Tomato ARF GTPase cont...

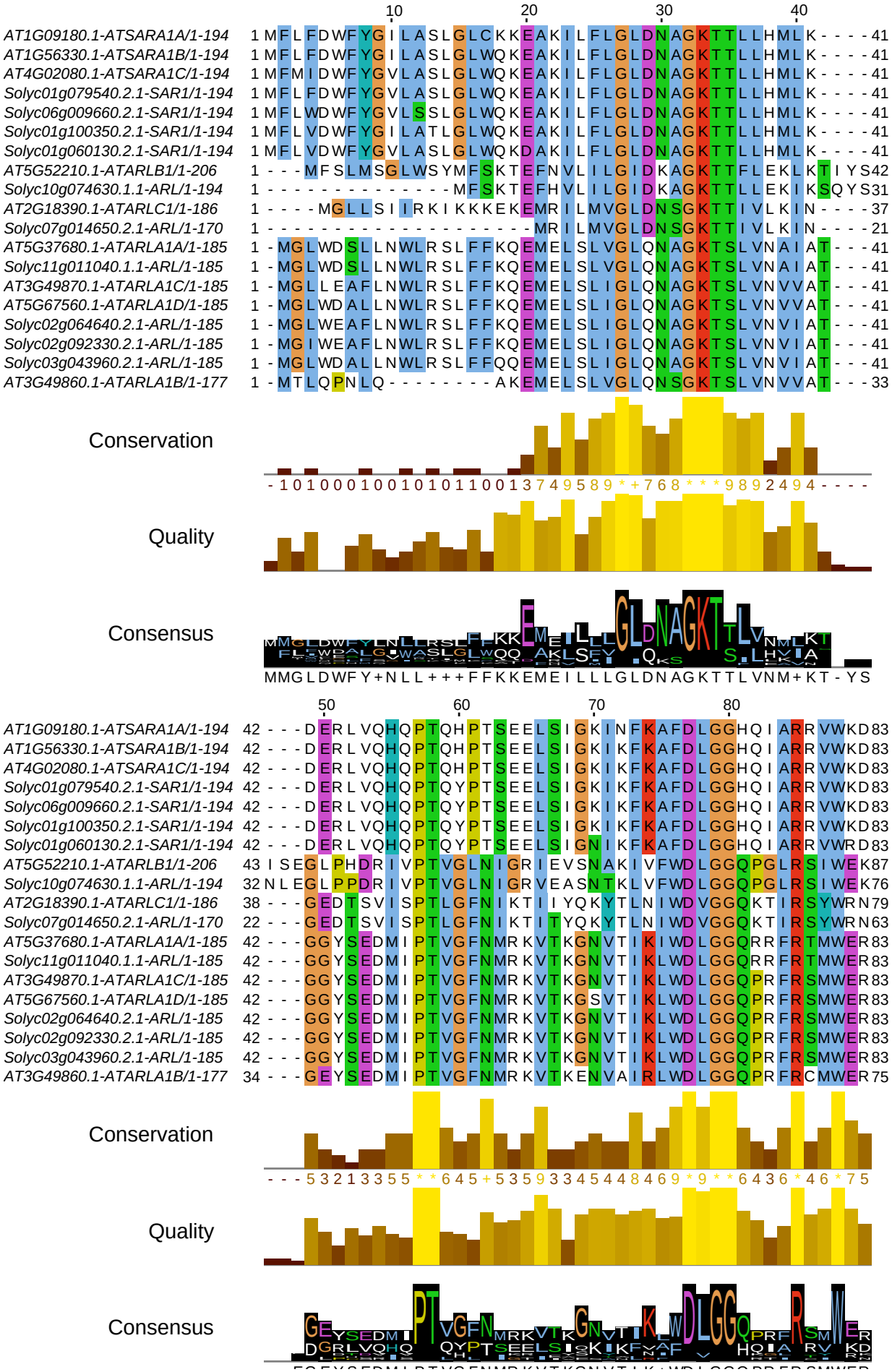


Fig.3.49 MSA of Arabidopsis and Tomato SAR & ARL GTPase

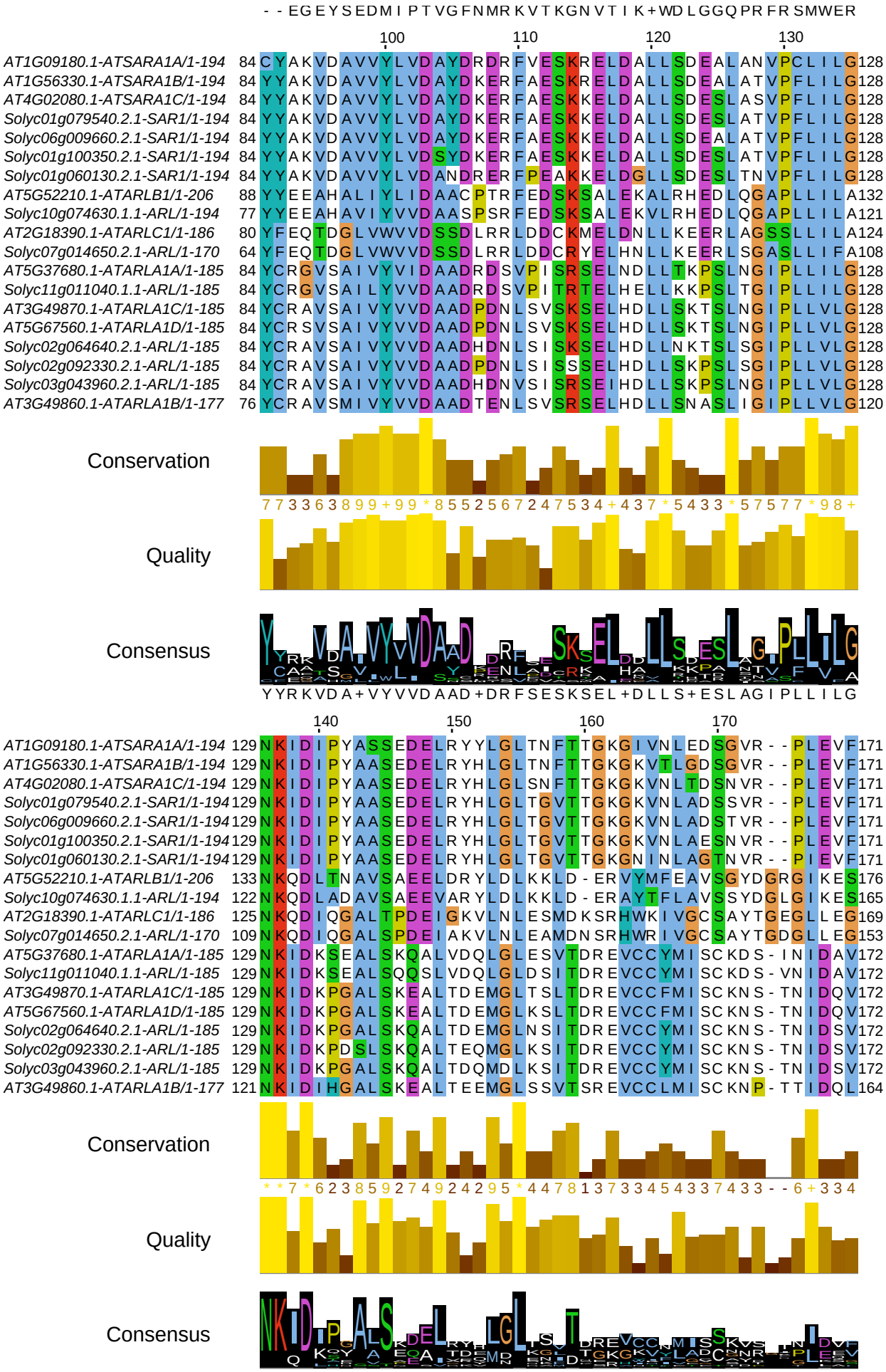


Fig.3.49 MSA of Arabidopsis and Tomato SAR & ARL GTPase cont...

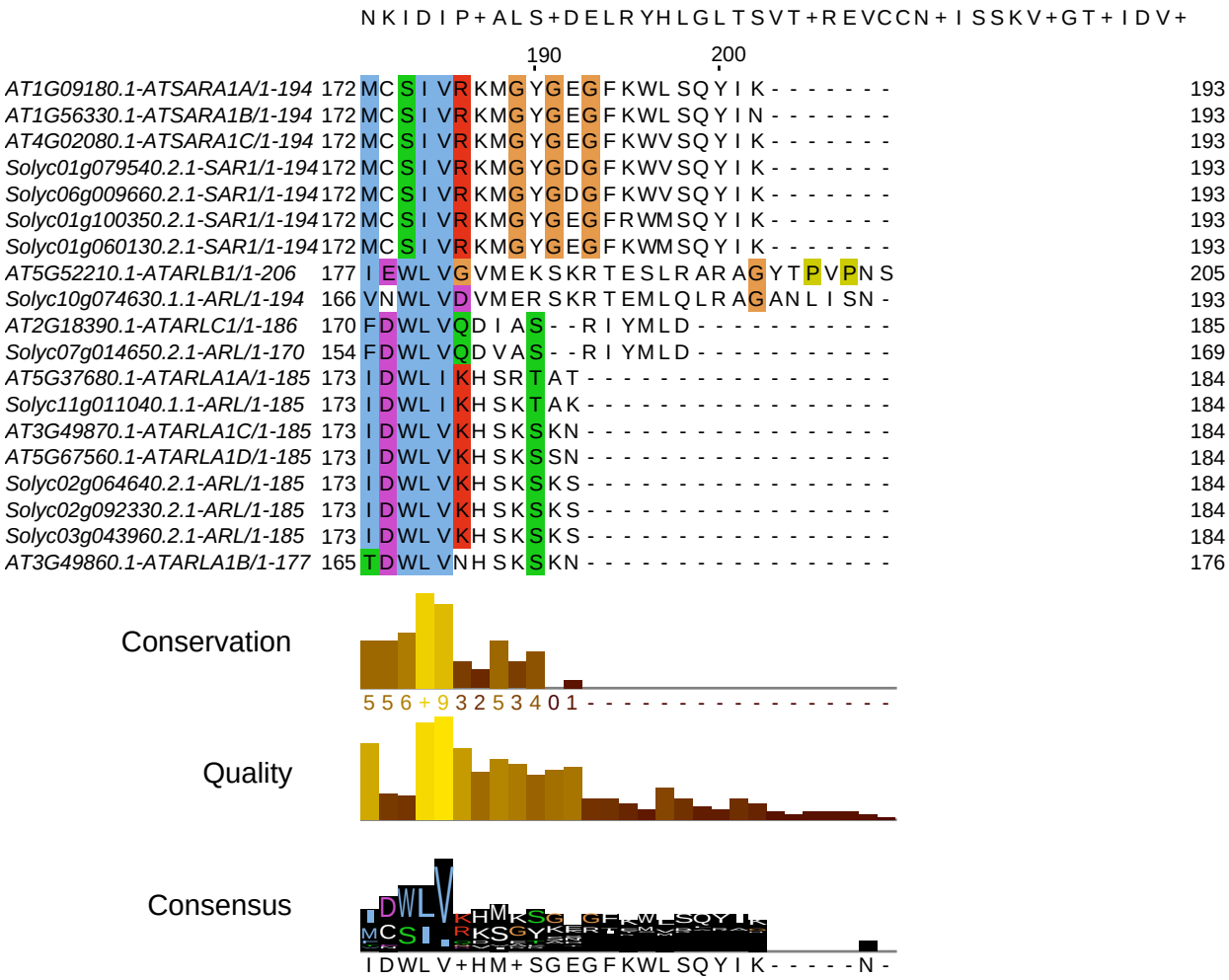


Fig.3.49 MSA of Arabidopsis and Tomato SAR & ARL GTPase cont...

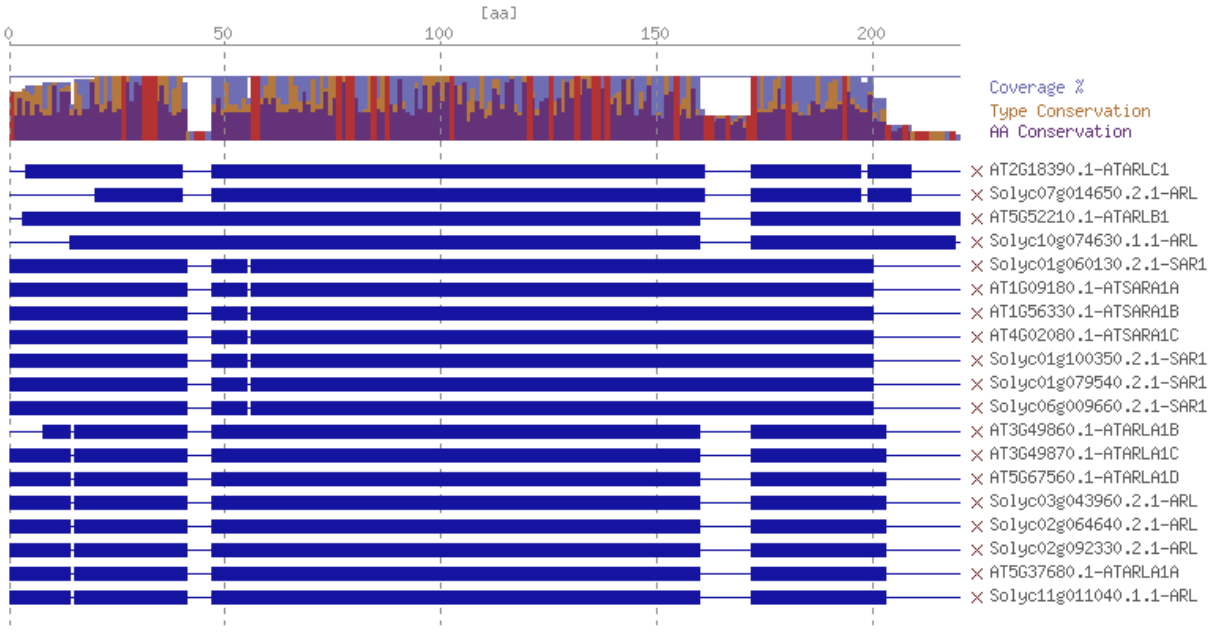


Fig. 3.50 Tomato and Arabidopsis SAR and ARL GTPase protein domain with Solyc Ids from SGN database (ITAG2.4) The fig. shows the domain architecture of Arabidopsis and tomato SAR and ARL GTPase aligned and grouped together. The Protein sequences were used to analyze the domain architecture in the SGN alignment analyzer. Fig also shows the percentage of sequence coverage, conservations plots and histogram for both.

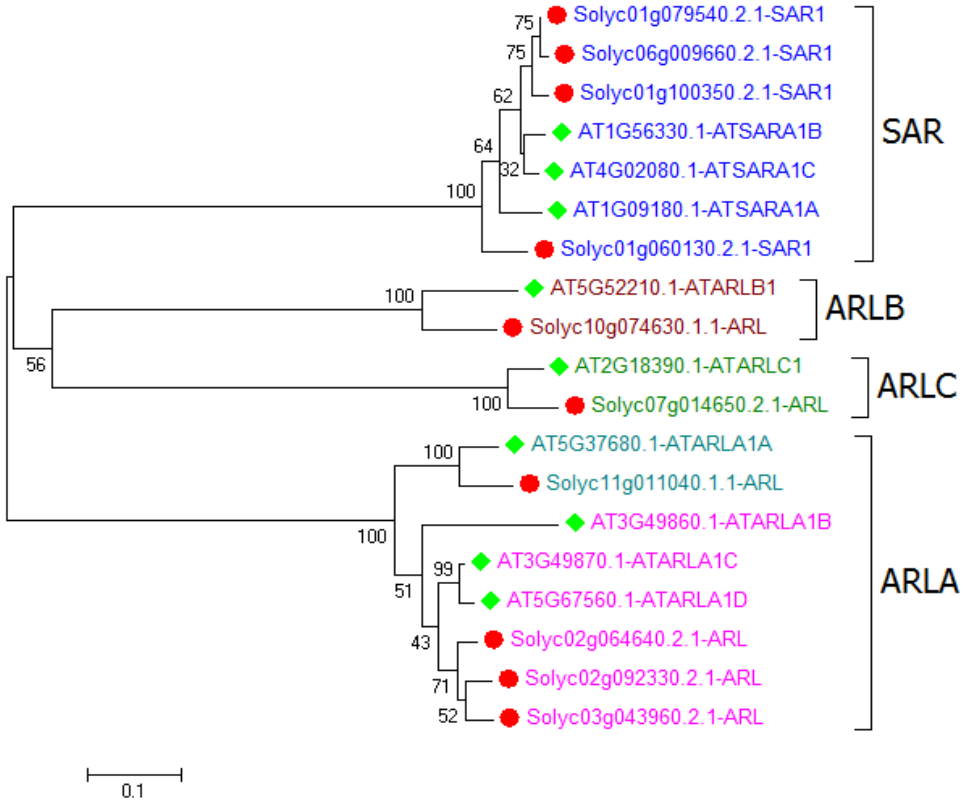


Fig. 3.51 Phylogenetic tree of At and Solyc SAR & ARL GTPase. The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 2.74 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Poisson correction method and are in the units of the number of amino acid substitutions per site. The analysis involved 14 amino acid sequences. Evolutionary analyses were conducted in MEGA6.

Table 3.26 List of Tomato ARL GTPase Solyc Ids from SGN ITAG2.4 Release

Sr.	Locus symbol	Chr	Annotation	Locus name	Region	Strand	NT	mRNA	Protein
	SAR								
1	Solyc01g060130	1	SAR-ADP-ribosylation factor-like protein 3 GTP-binding protein	Solyc01g060130.2	SL2.50ch01:69592187..69594251	+	2065	974	194
2	Solyc01g079540	1	SAR-ADP-ribosylation factor-like protein 3 GTP-binding protein	Solyc01g079540.2	SL2.50ch01:78611438..78620587	+	9,150	868	194
3	Solyc01g100350	1	SAR-ADP-ribosylation factor-like protein 3 GTP-binding protein	Solyc01g100350.2	SL2.50ch01:90412671..90415496	-	2,826	1,117	194
4	Solyc06g009660	6	SAR-ADP-ribosylation factor-like protein 3 GTP-binding protein	Solyc06g009660.2	SL2.50ch06:3608115..3612636	+	4,522	1,035	194
	ARL								
1	Solyc00g020990	0	ADP-ribosylation factor-like 8B	Solyc00g020990.1	SL2.50ch00:11911661..11913112	+	1,452	1,164	388
2	Solyc02g064640	2	ADP-ribosylation factor-like	Solyc02g064640.2	SL2.50ch02:35728695..35731426	-	2,732	821	185
3	Solyc02g092330	2	ADP-ribosylation factor-like	Solyc02g092330.2	SL2.50ch02:53426007..53430186	-	4,180	890	185
4	Solyc03g043960	3	ADP-ribosylation factor-like	Solyc03g043960.2	SL2.50ch03:7754312..7761697	-	7,386	999	185
5	Solyc07g014650	7	ADP-ribosylation factor-like	Solyc07g014650.2	SL2.50ch07:5030533..5038543	-	8,011	1,044	170
6	Solyc10g074630	10	ADP-ribosylation factor-like	Solyc10g074630.1	SL2.50ch10:58183787..58189509	+	5,723	582	194
7	Solyc11g011040	11	ADP-ribosylation factor-like	Solyc11g011040.1	SL2.50ch11:4089589..4094356	-	4,768	555	185

Putative ARL Solyc00g020990 was not included in the study because of longer ORF (388aa) and 'Solyc00' code which means it is yet to be mapped to the respective chromosome, but it had the IPR006689 for ARL GTPase. Future release and annotation may clarify the true identity of the sequence.

Table 3.27 List of Tomato RAB and ROP GDI - Solyc Ids from SGN ITAG2.4 Release

Sr.	Locus symbol	Chr	Annotation	Locus name	Region	Strand	NT	mRNA	Protein
1	Solyc01g105810	1	Rab GDP-dissociation inhibitor	Solyc01g105810.2	SL2.50ch01:93849291..93855176	+	5886	1675	446
2	Solyc02g082730	2	Rab GDP-dissociation inhibitor	Solyc02g082730.2	SL2.50ch02:46377419..46384215	+	6797	2,154	553
3	Solyc08g015860	8	Rab GDP dissociation inhibitor	Solyc08g015860.2	SL2.50ch08:6167425..6173736	+	6312	1,707	445
4	Solyc12g017570	12	Rab GDP dissociation inhibitor	Solyc12g017570.1	SL2.50ch12:6863578..6870564	+	6,987	1,335	445
1	Solyc04g014760	4	RHO GDP dissociation inhibitor	Solyc04g014760.2	SL2.50ch04:5004799..5008790	-	3,992	1,384	244
2	Solyc05g054110	5	RHO GDP dissociation inhibitor	Solyc05g054110.2	SL2.50ch05:64080673..64085207	+	4,535	970	234
3	Solyc09g072670	9	RHO GDP dissociation inhibitor 1	Solyc09g072670.2	SL2.50ch09:65280285..65292136	+	11,852	1,112	221
4	Solyc11g012270	11	RHO GDP dissociation inhibitor	Solyc11g012270.1	SL2.50ch11:5136039..5138781	-	2,743	783	261
5	Solyc12g098650	12	RHO GDPdissociation inhibitor 1	Solyc12g098650.1	SL2.50ch12:66087615..66089718	+	2,104	597	199

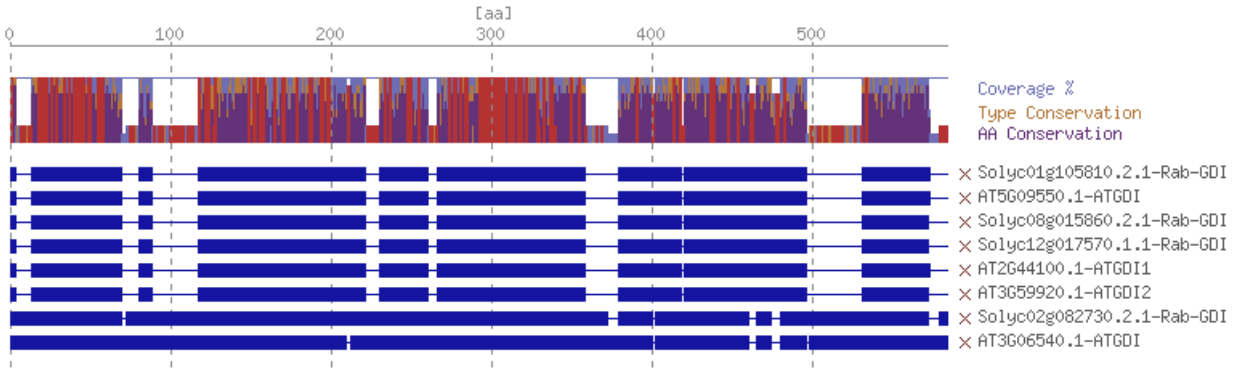


Fig. 3.52 Tomato and Arabidopsis RAB GDI protein domain with Solyc Ids from SGN database (ITAG2.4). The above fig. shows the domain architecture of Arabidopsis and tomato Rab GDI aligned and grouped together. The Protein sequences were used to analyze the domain architecture in the SGN alignment analyzer. Fig also shows the percentage of sequence coverage, conservations plots and histogram for both.

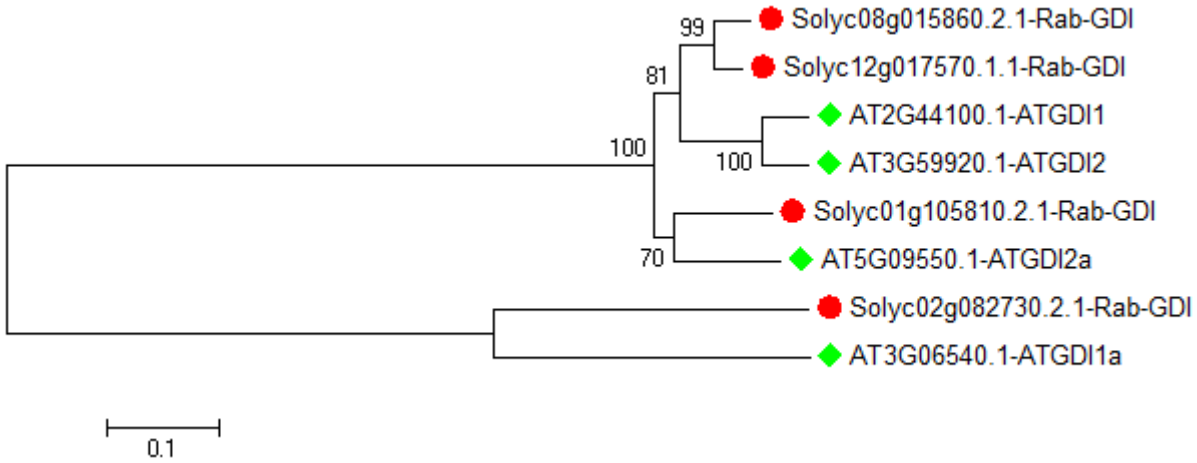


Fig. 3.53 Phylogenetic tree of Tomato and Arabidopsis RAB GDI
The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 2.06 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Poisson correction method and are in the units of the number of amino acid substitutions per site. The analysis involved 8 amino acid sequences. Evolutionary analyses were conducted in MEGA6.

3.18 Solyc Rab GEF and Rab GDI

As compared to the two Arabidopsis Rab GEF (AT3G19770 and AT5G09320.1) there was only one Rab GEF in tomato with solyc id Solyc01g096990.2.1 which is guanine nucleotide exchange factor Vps9 (Vacuolar sorting protein 9) with inter-pro id IPR013995. There are four Rab GDI and five ROP GDI in tomato (Table3.27). Fig. 3.52 shows the domain architecture of Tomato and Arabidopsis RAB GDI. The phylogenetic tree of At and Solyc RabGDI (Fig.3.53) shows that Solyc02g082730 falls with AtRabGDI1a and Solyc01g105810 falls with AtRabGDI2a. The other two Solyc08g015860 and Solyc12g017570 fall in the same cluster with the AtGDI1 and AtGDI2. Fig 3.54 shows the MSA of Tomato and Arabidopsis RAB GDI.

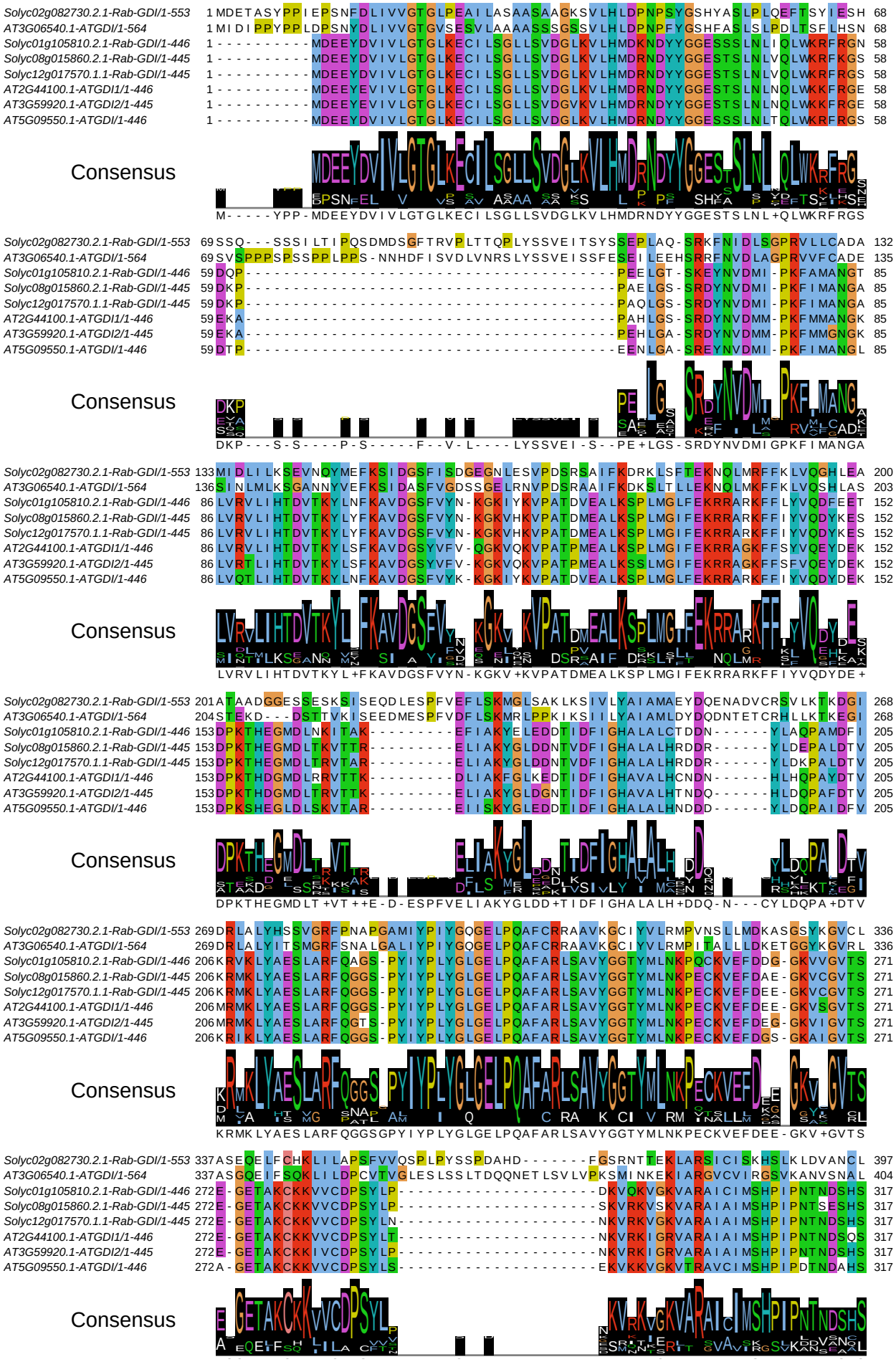


Fig.3.54 MSA of Arabidopsis and Tomato Rab GDI

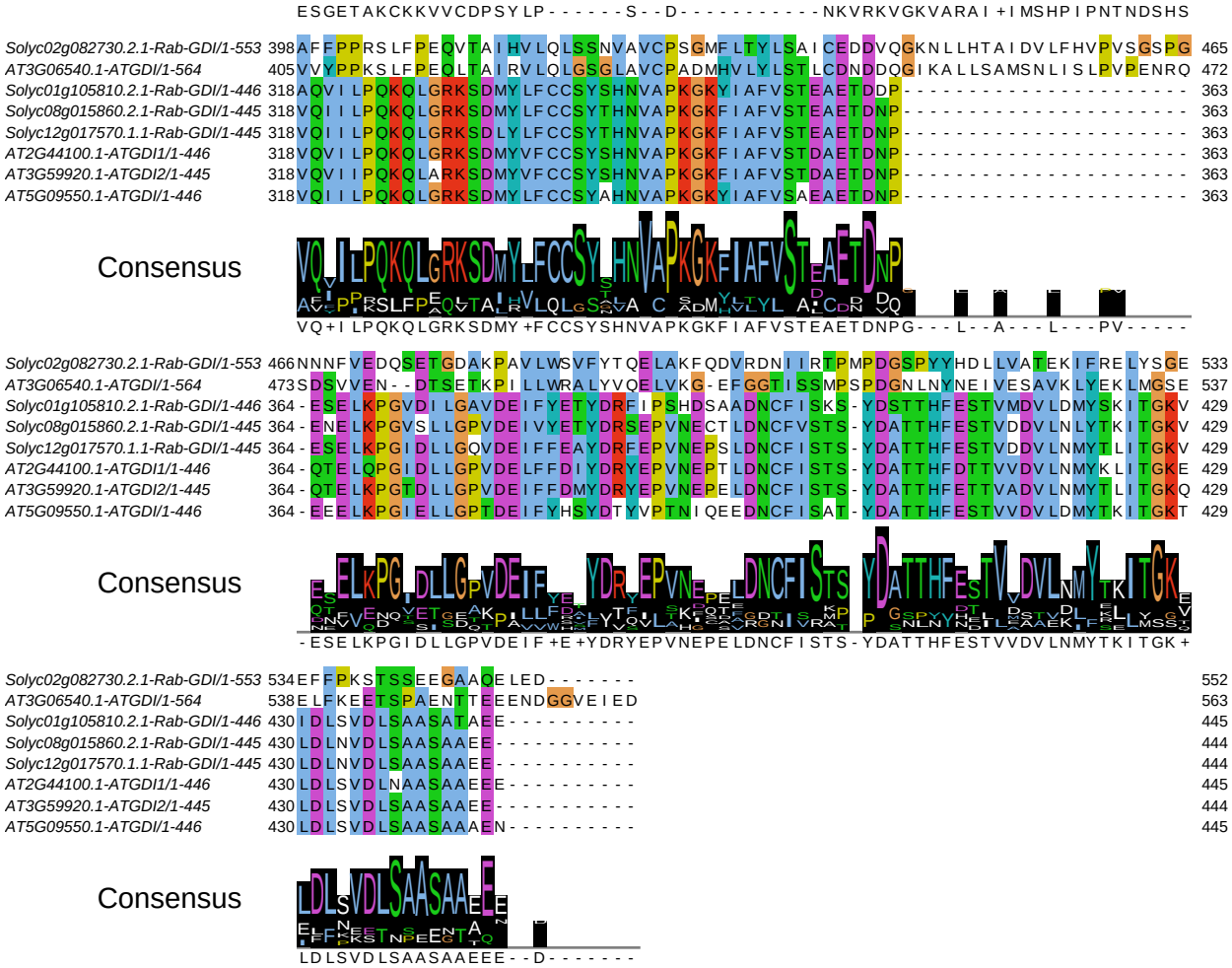


Fig.3.54 MSA of Arabidopsis and Tomato Rab GDI

Table 3.28 List of Tomato RAB-GAP, ROP-GAP, ARF-GAP and RAN-GAP Solyc Ids from SGN -ITAG2.4 Release

	Rab GAP	Name		Rho GAP	Name		ARF GAP	With ANKr PH		Ran GAP	Name
1	Solyc00g071590	Rab GAP/TBC	1	Solyc01g008770	Rho GAP12	1	Solyc02g024020	Arf GAP ANKr PH3	1	Solyc01g079680	Ran GAP
2	Solyc01g101090	Rab GAP/TBC	2	Solyc01g096360	Rho GAP2	2	Solyc03g006610	Arf GAP ANKr PH1	2	Solyc09g065190	Ran GAP1
3	Solyc02g014450	Rab GAP/TBC1 fm5	3	Solyc03g093430	Rho GAP1	3	Solyc03g098500	Arf GAP ANKr-PH1			
4	Solyc02g071420	Rab GAP/TBC	4	Solyc04g076140	Rho GAP12	4	Solyc03g118260	Arf GAP ANKr-PH3			
5	Solyc03g058470	Rab GAP/TBC1 fm15	5	Solyc06g084450	Rho GAP2	5	Solyc04g008660	Arf GAP ANKr-PH1			
6	Solyc03g082590	Rab GAP/TBC	6	Solyc09g009970	Rho GAP2	6	Solyc04g009560	Arf GAP ANKr-PH1			
7	Solyc04g009560	Rab GAP/TBC	7	Solyc09g074340	Rho GAP2	7	Solyc05g023750	Arf GAP ANKr-PH3			
8	Solyc05g053710	Rab GAP/TBC1fm13	8	Solyc11g064870	Rho GAP22	8	Solyc07g063550	Arf GAP ANKr-PH1			
9	Solyc06g008250	Rab GAP/TBC1fm17	9	Solyc11g068520	Rho GAP2	9	Solyc08g043140	Arf GAP ANKr-PH3			
10	Solyc06g053190	Rab GAP/TBC				10	Solyc10g006850	Arf GAP ANKr-PH1			
11	Solyc07g008000	Rab GAP/TBC1fm15				11	Solyc10g076990	Arf GAP ANKr-PH1			
12	Solyc07g008840	Rab GAP/TBC1fm5				12	Solyc12g042170	Arf GAP ANKr-PH1			
13	Solyc07g049580	Rab GAP/TBC1fm2				13	Solyc12g042180	Arf GAP ANKr-PH3			
14	Solyc07g064230	Rab GAP/TBC1fm17					**ARF GAP**				
15	Solyc09g007160	Rab GAP/TBC1fm9b				1	Solyc00g023260	Arf GAP1			
16	Solyc09g066420	Rab GAP/TBC1fm15				2	Solyc08g005070	Arf GAP3			
17	Solyc09g092070	Rab GAP/TBC1fm13				3	Solyc08g067930	Arf GAP			
18	Solyc10g006440	Rab GAP/TBC1fm17				4	Solyc08g076920	Arf GAP3			
19	Solyc11g005010	Rab GAP/TBC1fm13				5	Solyc09g010520	Arf GAP1			
20	Solyc12g005220	Rab GAP/TBC1 fm17				6	Solyc10g084690	Arf GAP1			
21	Solyc12g005930	Rab GAP/TBC1fm2									
22	Solyc12g009610	Rab GAP/TBC1fm15									

Solyc03g058500 not included in the study 77 aa length Solyc00g117650 not included in the study 82 aa length **Ankr:** Ankyrin repeat **PH:** Pleckstrin Homology

Table 3.29 Blast hit Results of tomato Rab GAP with Solyc IDs (ITAG2.4)

AtGAP Group	Gene Name	AGI Code	Hits in SGN Solyc Ids	Annotation Domain information	Score
AtGYPA	AtGYPA1a	AT2G30710.1	Solyc11g005010.1.1	TBC1 domain family member 13	666
	AtGYPA1b	AT4G13730.1	Solyc09g092070.2.1	TBC1 domain family member 13	570
	AtGYPA1c	AT1G04830.1	Solyc09g092070.2.1	TBC1 domain family member 13	553
AtGYPB1	AtGYPB1a	AT2G20440.1	Solyc06g008250.2.1	TBC1 domain family member 17	578
			Solyc09g066420.2.1	TBC1 domain family member 15	566
	AtGYPB1b	AT4G28550.1	Solyc06g008250.2.1	TBC1 domain family member 17	578
			Solyc09g066420.2.1	TBC1 domain family member 15	578
	AtGYPB1c	AT4G27100.1	Solyc10g006440.2.1	TBC1 domain family member 17	659
			Solyc07g064230.2.1	TBC1 domain family member 17	611
			Solyc12g005220.1.1	TBC1 domain family member 17	598
	AtGYPB1d	AT5G54780.1	Solyc10g006440.2.1	TBC1 domain family member 17	662
			Solyc07g064230.2.1	TBC1 domain family member 17	622
			Solyc12g005220.1.1	TBC1 domain family member 17	619
			Solyc06g008250.2.1	TBC1 domain family member 17	518
			Solyc09g066420.2.1	TBC1 domain family member 15	520
	AtGYPB1e	AT2G43490.1	Solyc03g058470.1.1	TBC1 domain family member 15	574
	AtGYPB1f	At3g57590	F box Protein	ATGYPB1f is F box Protein	
AtGYPB2	AtGYPB2a	At5g52590	Obsolete	AGI not found (AT5G52590.1) in TAIR10	
		At5g52580	Solyc12g009610.1.1	TBC1 domain family member 15	
	AtGYPB2b	AT5G41940.1	Solyc03g082590.2.1	TBC1 domain family member 15	610
			Solyc06g053190.2.1	GTPase activating-like protein	573
	AtGYPB2c	AT5G53570.1	Solyc03g082590.2.1	TBC1 domain family member 15	595
			Solyc06g053190.2.1	GTPase activating-like protein	591
	AtGYPB2d	AT3G49350.1	Solyc03g082590.2.1	TBC1 domain family member 15	643
			Solyc06g053190.2.1	GTPase activating-like protein	626
	AtGYPB2e	AT5G24390.1	Solyc03g082590.2.1	TBC1 domain family member 15	579
			Solyc06g053190.2.1	GTPase activating-like protein	571
AtGYPC	AtGYPC1a	AT3G07890.1	Solyc12g005930.1.1	TBC1 domain family member 2	677
			Solyc07g049580.2.1	TBC1 domain family member 2	673
	AtGYPC1b	AT3G55020.1	Solyc04g009560.2.1	TBC1 domain family member 8B	946
			Solyc09g007160.2.1	TBC1 domain family member 9B	805
	AtGYPC1c	AT2G39280.1	Solyc04g009560.2.1	TBC1 domain family member 8B	875
			Solyc09g007160.2.1	TBC1 domain family member 9B	730
	AtGYPC1d	AT2G37290.1	Solyc09g007160.2.1	TBC1 domain family member 9B	786
AtGYPD	AtGYPD1a	AT3G02460.1	Solyc01g101090.2.1	TBC1 domain family member CG11727	568
	AtGYPD1b	AT5G15930.1	Solyc01g101090.2.1	TBC1 domain family member CG11727	577

Parallel BLAST hits in SGN with Solyc IDs and annotation with domain information

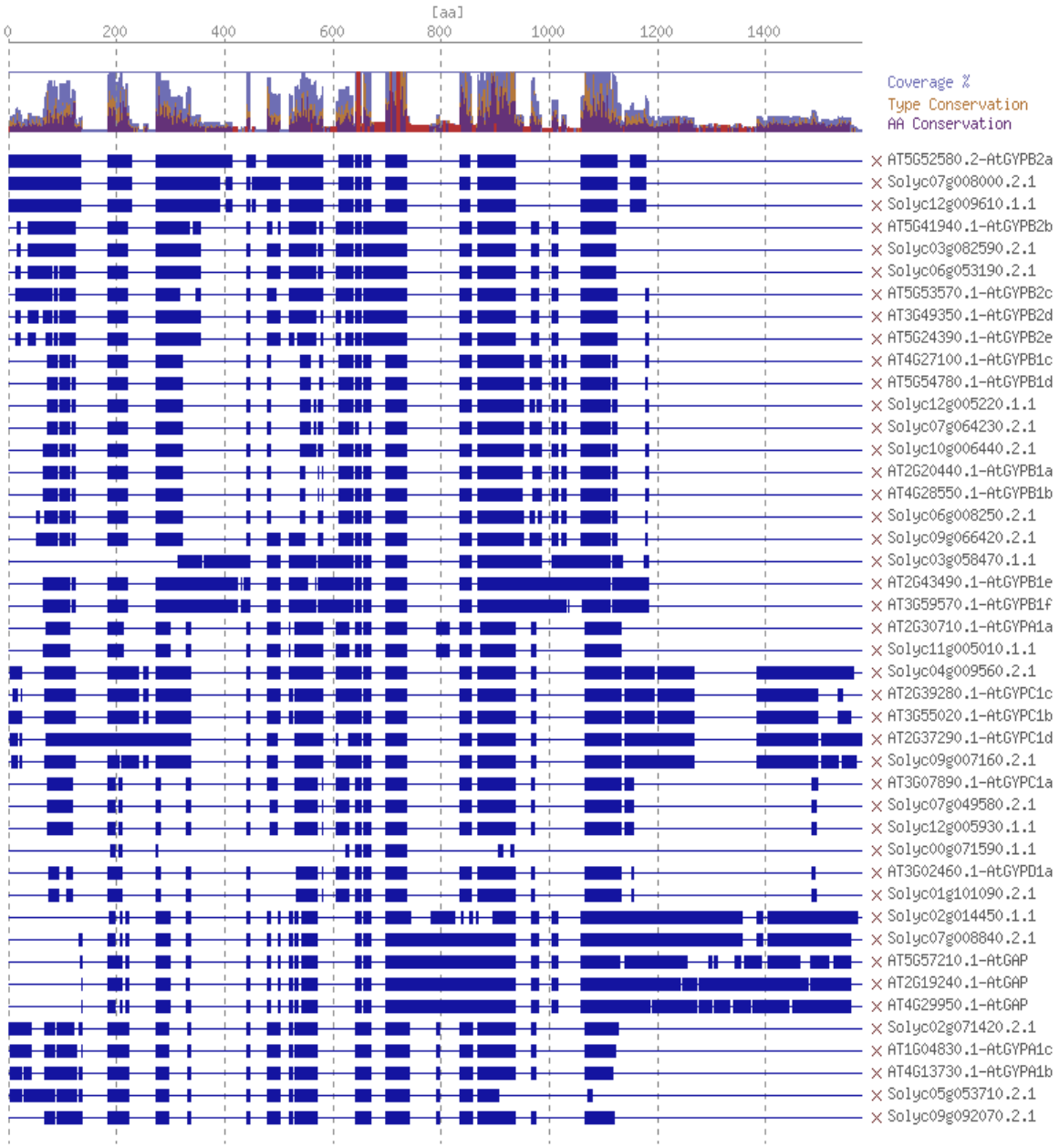


Fig.3.55 Tomato and Arabidopsis Rab GAP/GYP protein domain with Solyc Ids from SGN database (ITAG2.4). The fig. shows the domain architecture of Arabidopsis and tomato Rab GAP/GYP aligned and grouped together. The Protein sequences were used to analyze the domain architecture in the SGN alignment analyzer. Fig also shows the percentage of sequence coverage, conservations plots and histogram for both.

3.19 Solyc Rab GAP

A list of Tomato RAB, ROP, ARF and RAN GAP Solyc Ids from SGN -ITAG2.4 are shown in table 3.28. Blast hit Results of tomato Rab GAP with Solyc IDs (ITAG2.4) using Arabidopsis RabGAP as query are listed in table 3.29. In Arabidopsis the there are 22 reported RabGAP. A total of 24 RabGAPs were identified in tomato, out of these 24 only 22 Rab GAP sequences were included in the present study for analysis.

Tomato RabGAP with Solyc ids Solyc03g058500 (77 aa) and Solyc00g117650 (82aa) were not included in the study because of incomplete sequences. The domain architectures of Tomato and Arabidopsis Rab GAP/GYP proteins were analyzed in the SGN alignment analyzer and were grouped together as shown in fig.3.55. Using the 22 Arabidopsis and 22 tomato RabGAPs MSA was generated and a comparative phylogenetic tree of At v/s Solyc Rab GAP was generated (Fig. 3.56). The results of phylogeny member classification and groups are discussed below

Clade1: GYPB1

Clade1 consist of the RabGYPB1/RabGAPB1. The B1 group has 6 Arabidopsis and 6 Tomato members clustered together. In the B1cluster the single node of AtRabGAPB1a and AtRabGAPB1b fall together with tomato Solyc06g008250 and Solyc09g066420. The other node has three tomato members *viz.*, Solyc07g064230, Solyc10g006440 and Solyc12g005220 falling together with the AtRabGAPB1c and B1d. There is one single tomato member Solyc03g058470 belonging to the sub group of At RabGAPB1e and B1f.

Clade2: GYPB2

In clade2 of AtRabGAPB2 the cluster had 5 Arabidopsis and 4 tomato sequences. The parallel homologs of Arabidopsis AtRabGAPB2a in tomato are Solyc07g008000 and Solyc12g009610 falling in the same node. The other B2 group members of AtRabGAPB2b, B2c, B2d and B2e are all clustered together with Solyc03g082590 and Solyc06g053190 of tomato member sequences.

Clade3: GYPA

The clade3 consist RabGAPA group of 3 members of Arabidopsis and 4 tomato members clustered together. AtRabGAPA1a fall together with the Solyc11g005010 in a single node, likewise AtRabGAPA1b falls with Solyc05g053710. There are two tomato members in the sub group of RabGAPA1c with Solyc02g071420 and Solyc09g092070 lying together with AtRabGAPA1c.

Clade4: GYPD

There is a single member of tomato in the D subgroup where in Solyc01g101090 falls with AtRabGAPD1a. AtGYPD1b (AT5G15930) was not included in the study.

Clade5: GYPC

Clade5 consists of RabGAPC group with 4 Arabidopsis and 4 identified tomato homologs. AtRabGAPC1a has two parallel tomato members Solyc12g005930 and Solyc07g049580 in the same clustered node. AtRabGAPC1b has a single

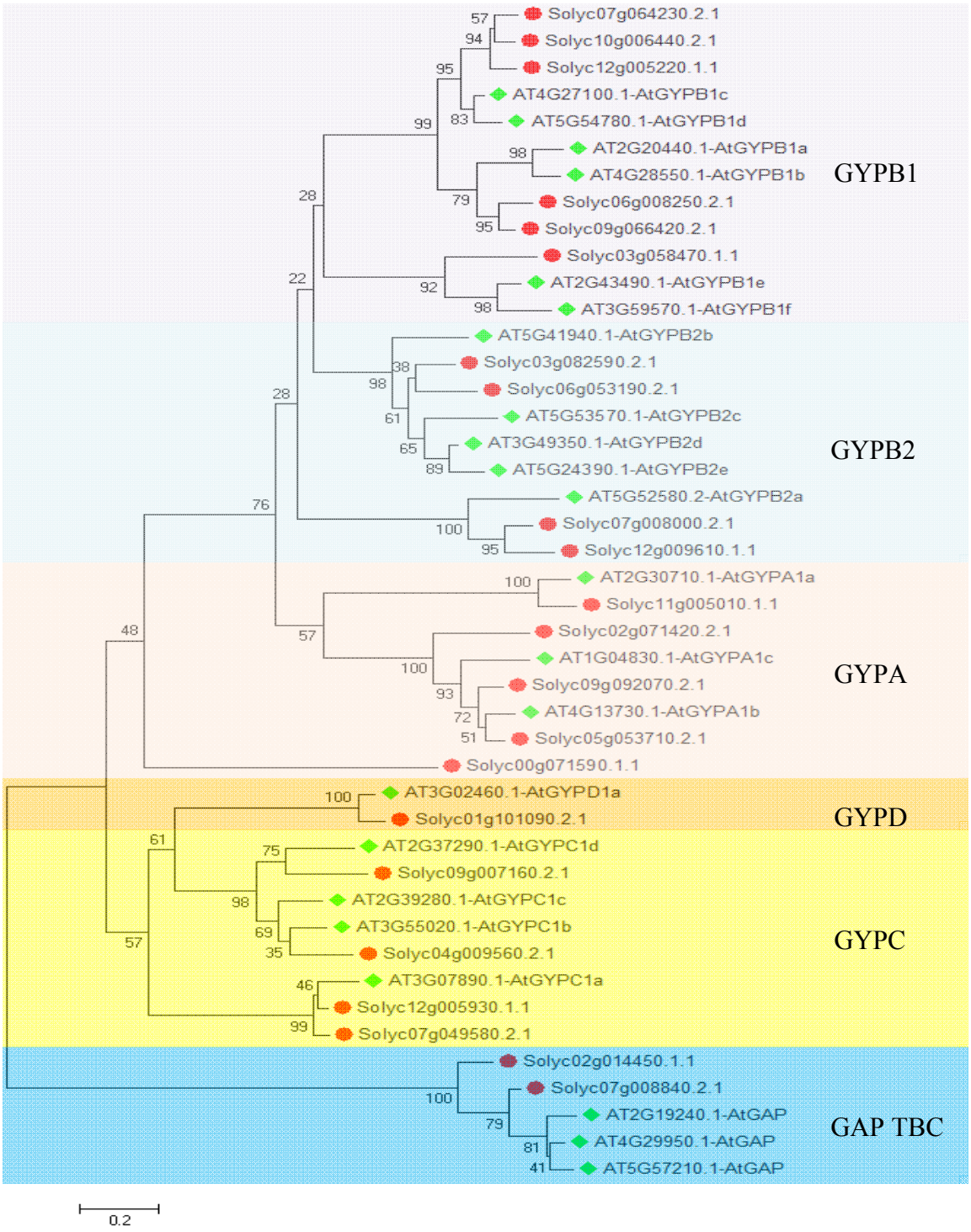


Fig. 3.56 Phylogenetic tree of Arabidopsis and Tomato Solyc Rab-GAP (SGN-ITAG 2.4). The phylogenetic tree of Arabidopsis and Tomato Rab GAP/GYP was inferred using the Neighbor-Joining method. The tress shows the GYPA, B, C and D class of AtGAP/GYP and GAP TBC domain as well. The optimal tree with the sum of branch length = 11.72 is shown. The percentages of replicate trees with bootstrap test (1000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Poisson correction method and are in the units of the number of amino acid substitutions per site. The analysis involved 44 amino acid sequences. Evolutionary analyses were conducted in MEGA6. For the MSA analysis see the supplemental files.

Solyc04g009560 tomato member and likewise in the case of AtRabGAPC1d is lying with single tomato member with sequence id Solyc09g007160.

Clade6: GAP-TBC

The last clade6 of RabGAPs consist of total 5 members with 3 in Arabidopsis and 2 in tomato. These Rab GAPs have the TBC domain and annotation was checked in TAIR and SGN database inorder to include them in the present analysis. The three Arabidopsis RabGAPs with AGI codes At2G19240, At4G29950 and At5G57210 fall in the same distinct cluster with two of tomato sequences with ids Solyc02g014450 and Solyc07g008840. This completes the analysis, classification and grouping of the tomato RabGAPs. The MSA analysis is in the supplemental files in the cd.

Along with the tomato RabGAP analysis the other GAPs were also identified for other small GTPase members like RAN GAP, ROP/RHO GAP and ARF GAP (Table 3.28). The analysis of these GAPs is not so simple as the sequence variation is quite high and other issue includes the false positive identification because of the computer generated automated annotations. The analysis revealed there are atleast 2 RAN GAPs in tomato with sequence ids Solyc01g079680 and Solyc09g065190.

In case of tomato RHO/ROP GTPases a total of 9 ROP GAPs were identified as compared to six reported ROP GAPs in Arabidopsis.

The complex analysis of ARF GAP revealed that in tomato there are at least 19 members of ARF GAP altogether. In Arabidopsis a total of 15 members have been reported. The basic set of tomato ARF GAPs consist of 6 member sequences as shown in table 3.28. The ARF GAPS with the Ankr- (Ankyrin rich repeat) and Pleckstrin Homology (PH) domain were also identified and in tomato at present there are 13 members lying in this group. In Arabidopsis a different set of nomenclature for ARF GAP has been used as compared to the RabGAPs. The sequences identified with the GAP specific domains related to ARF are termed as ARF GAP Domain containing proteins (AGD) with 15 members in Arabidopsis. This is the first report of ARF GAPs in tomato. The MSA analysis and phylogenetic analysis and further sub group classification is not included in the thesis likewise the comparative analysis of GAPs in other Solanaceae species (potato, tobacco, petunia, chilli peppers) are not included in the thesis considering the volume, but will be published in the forthcoming research papers.

3.20 Conclusion

The Rab GTPase genes of Arabidopsis were obtained from the TAIR database. These sequences were used to search tomato Rab GTPases from TIGR DFCI TGI, SGN and TED databases. Sixty genes coding Rab GTPases in tomato were identified by *in silico* analysis. The protein sequences were grouped into 8 different functional classes ranging from Rab1, 2, 5, 6, 7, 8, 11 and Rab18. There are nine RabD, four Rab B, five members in RabF and Rab H group, seven and eight members in Rab G and RabE and three in Rab C group. The highest numbers of genes were found in RabA/11 class. The family specific and sub family specific motifs of the tomato Rab proteins were inferred by the inter-pro scan and the secondary structure revealed that all the tomato Rabs identified were having these Rab GTPase specific motifs. After MSA the phylogenetic tree was constructed which revealed that each gene belonging to the group falls together in the same cluster of Rab groups, this was further validated by constructing a phylogenetic tree with the Arabidopsis Rab GTPase genes which confirmed that the tomato Rab GTPase genes were also falling in the same cluster of Arabidopsis Rab groups. The expression profiles were generated from the TED database to find the expression pattern of these Rab genes in fruit ripening related manner. The results revealed that the target LeRab11 is expressed in early stages of fruit ripening and the expression goes down at the later stages of fruit ripening particularly in red ripe fruits.

The other elements involved in vesicle trafficking apart from Rab GTPases were also identified using the same methods. The results showed that there are 13 ARF GTPases, eighteen ARL GTPases, seven SAR GTPases, eight ROP GTPases, and a total of four out of nine curated and confirmed RAN GTPases in tomato. The Rab GTPase regulators like GAP, GDI and GEF were also identified. The results revealed that there are 23 RAB GAP, two Rab GEF and a total of six RAB GDI. The MSA and the phylogenetic tree for all of these proteins involved in the intracellular vesicle trafficking were generated using Arabidopsis proteins of the corresponding family as reference in order to validate the results.

The release of tomato WGS lead to availability of a more concrete resource for mining, identification, classification and annotating the tomato Rab GTPase genes. Although issues were found with bulk data retrieval, individual sequences were visually inspected and then used for analysis. The new Solyc Ids (ITAG2.4) were

found and subjected to the multiple sequence analysis following the same pathway used for the analysis in case of TIGR TC sequences. The results of MSA and phylogeny reveal that different Rab GTPase fall in distinct 8 classes with highest numbers found in RabA/Rab11 group. Total Rab GTPase with Solyc IDs screened and verified through the database (SGN-ITAG2.4) are listed below

Rab GTPase Class	Arabidopsis ITAG	Tomato Solyc	RabA GTPase Group	Arabidopsis ITAG	Tomato Solyc
Rab1	4	5	RabA1	9	7
Rab2	3	5	RabA2	4	6
Rab5	3	4	RabA3	1	1
Rab6	5	3	RabA4	5	5
Rab7	8	4	RabA5	5	5
Rab8	5	5	RabA6	2	1
Rab11	26	25			
Rab18	3	4			
Total	**57**	**55**	**Total**	**26**	**25**

The numbers in the initial analysis of Rab GTPase with Solyc ids and the screening of current database varied. The Solyc Ids with incomplete sequences or which were not Rabs were not included in the analysis.

Thus to conclude the results of the *in silico* analysis have been quiet reassuring and this is the first report of the classification of all tomato Rab GTPase with their regulators (Rab GAP,GDI, GEF) and also of other small GTPases like ARF, ARL, SAR, RAN and ROP GTPase and their regulators as summarised below

GTPase	Arabidopsis	Tomato	GTPase	Arabidopsis	Tomato
RAN	4	4	ARF A	6	7
ROP	11	10	ARF B	3	4
ARF	12	12	ARF C	1	1
ARL	6	6	ARF D	2	0
SAR	3	4	ARL A	4	4
RAB GEF	2	1	ARL B	1	1
ROP GEF	2	2	ARL C	1	1
RAB GDI	4	4	RabGAP A	3	5
ROP GDI	4	5	RabGAP B	11	10
RAB GAP	22	22	RabGAP C	4	4
RAN GAP	2	2	RabGAP D	2	1
ROP GAP	6	9	RabGAP-TBC	3	2
ARF GAP	15	19			

4. Molecular and Phenotypic Characterization of Tomato *LeRab11a* Antisense Lines

4.1 Molecular Characterization of Tomato *LeRab11a* Antisense Lines

4.1.1 Analysis of progeny of transgenic R_3T_1 lines for the Mendelian segregation ratio.

The seeds from the nine lines of the *LeRab11a* antisense (R_3T_1) generation were sown. From each of the nine lines, 24 seeds were sown and the leaf samples from each of the transgenic lines of the progeny of the R_3T_1 line were used for the DNA extraction. The presence of the antisense *LeRab11a* transgene was confirmed by PCR using the gene specific primers. The wild type *Ailsa craig* (AC++) was used as negative control for the experiment. The agarose gel electrophoresis was done for the PCR products. The DNA ladder (Molecular marker 1Kb) was loaded in the first lane, along with the 10 µl of the PCR product followed by the negative control. The band of expected size of 0.96 Kb was observed. The progeny of the first line of R3 T1 were named from P1-1 to P1-24 and likewise for line P2 from P2-1 to P2-24 and from P9-1 to P9-24 and for the wild type, control plants the progeny was named from C1-24. The results are shown in the following figures from Fig. 4.1 to Fig 4.7.

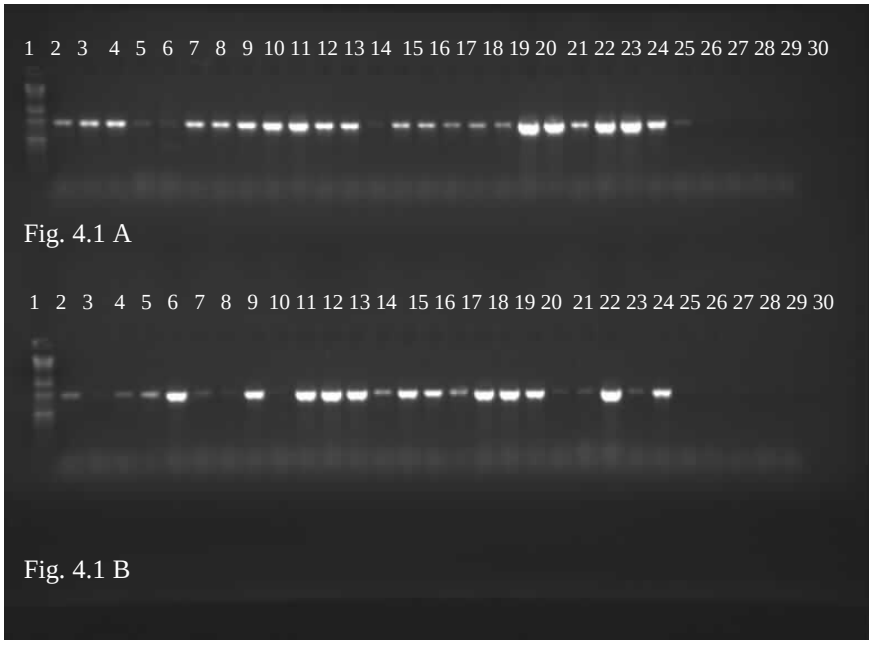


Fig: 4.1 Agarose gel electrophoresis of the PCR products from the progeny P1 (P1-1 to P1-24) and P2 (P2-1 to P2-24) of the R_3T_1 lines.

4.1 A **Lane 1**: 1 Kb DNA ladder
Lane 2-25: P1-1; P1-2; P1-3; P1-4; P1-5; P1-6; P1-6; P1-7; P1-8; P1-9: P1-10; P1-11; P1-12; P1-13; P1-14; P1-15; P1-16; P1-17; P1-18; P1-19; P1-20; P1-21; P1-22; P1-23; P1-24
Lane 26-30: C-1; C-2; C-3; C-4; C-5

4.1 B **Lane 1**: 1 Kb DNA ladder
Lane 2-25: P2-1; P2-2; P2-3; P2-4; P2-5; P2-6; P2-6; P2-7; P2-8; P2-9: P2-10; P2-11; P2-12; P2-13; P2-14; P2-15; P2-16; P2-17; P2-18; P2-19; P2-20; P2-21; P2-22; P2-23; P2-24
Lane 26-30: C-6; C-7; C-8; C-9; C-10

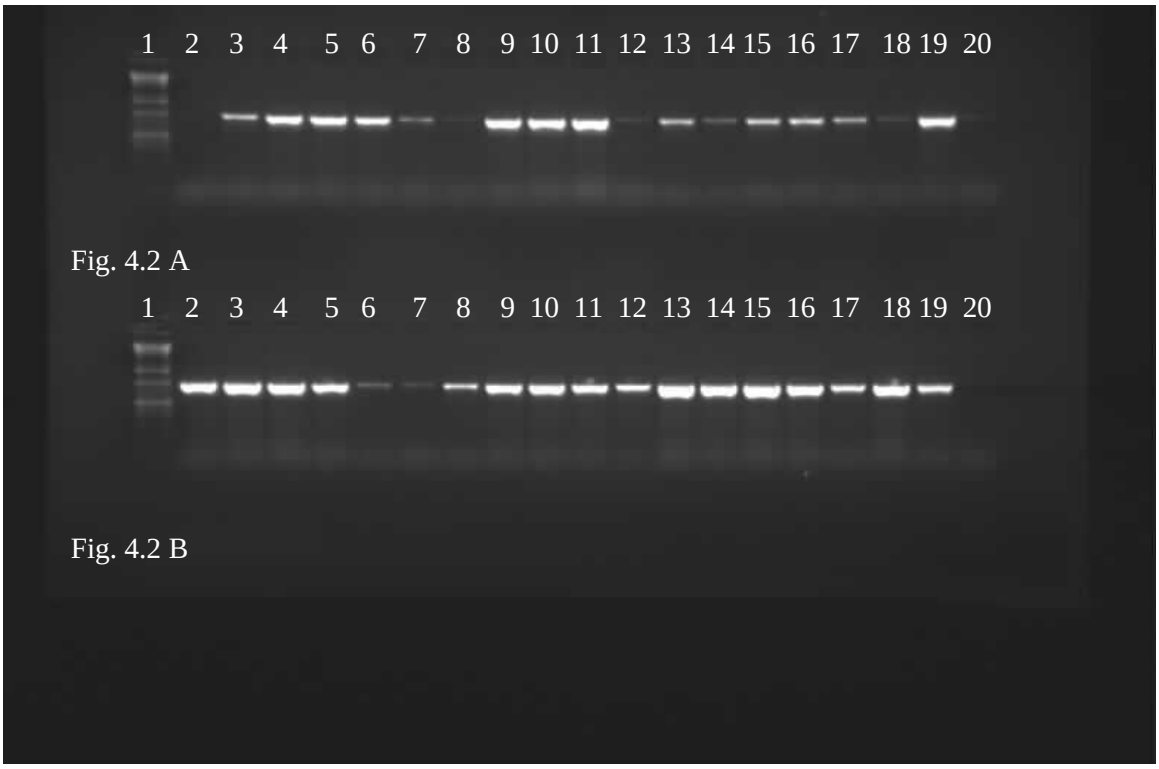


Fig. 4.2 Agarose gel electrophoresis of the PCR products from the progeny P3 (P3-1 to P3-24) and P4 (P4-1 to P4-12) of the R_3T_1 lines.

4.2 A

Lane1: 1 Kb DNA ladder

Lane 2-20: P3-1; P3-2; P3-3; P3-4; P3-5; P3-6; P3-6; P3-7; P3-8; P3-9: P3-10; P3-11; P3-12; P3-13; P3-14; P3-15; P3-16; P3-17; P3-18; P3-19

4.2 B

Lane1: 1 Kb DNA ladder

Lane 2-6: P3-20; P3-21; P3-22; P3-23; P3-24 **Lane 7**: C-11

Lane 8-19: P4-1; P4-2; P2-3; P4-4; P4-5; P4-6; P4-6; P4-7; P4-8; P4-9: P4-10; P4-11; P4-12.

Lane 20: C-11

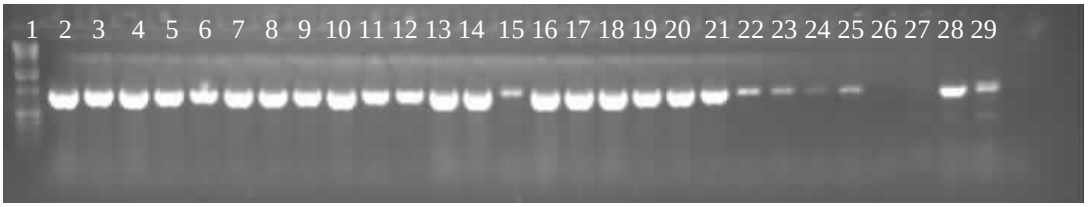


Fig: 4.3 Agarose gel electrophoresis of the PCR products from the progeny P4 (P4-13 to P4-24) and P5 (P5-1 to P5-12) of the R_3T_1 lines.

Lane1: 1 Kb DNA ladder

Lane 2-13: P4-13; P4-14; P2-15; P4-16; P4-17; P4-18; P4-19; P4-20; P4-21; P4-22; P4-23; P4-24

Lane 14-25: P5-1; P5-2; P5-3; P5-4; P5-5; P5-6; P5-6; P5-7; P5-8; P5-9: P5-10; P5-11; P5-12.

Lane 26-27: -ve control, C-13; C-14

Lane 28-29: +ve control

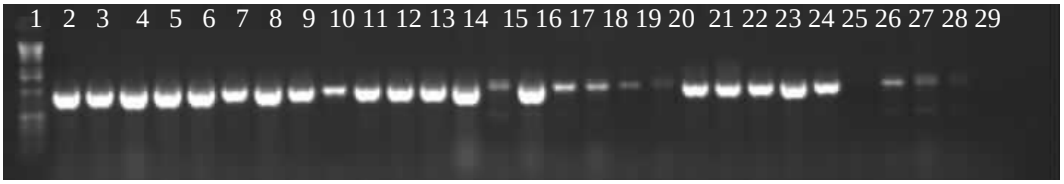


Fig: 4.4 Agarose gel electrophoresis of the PCR products from the progeny P5 (P5-13 to P5-24) and P6 (P6-1 to P6-12) of the R_3T_1 lines.

Lane1: 1 Kb DNA ladder

Lane 2-13: P5-13; P5-14; P5-15; P5-16; P5-17; P5-18; P5-19; P5-20; P5-21; P5-22; P5-23; P5-24

Lane 14-25: P6-1; P6-2; P6-3; P6-4; P6-5; P6-6; P6-7; P6-8; P6-9: P6-10; P6- 11; P6-12.

Lane 26-27: +ve control

Lane 28-29: -ve control, C-15; C-16

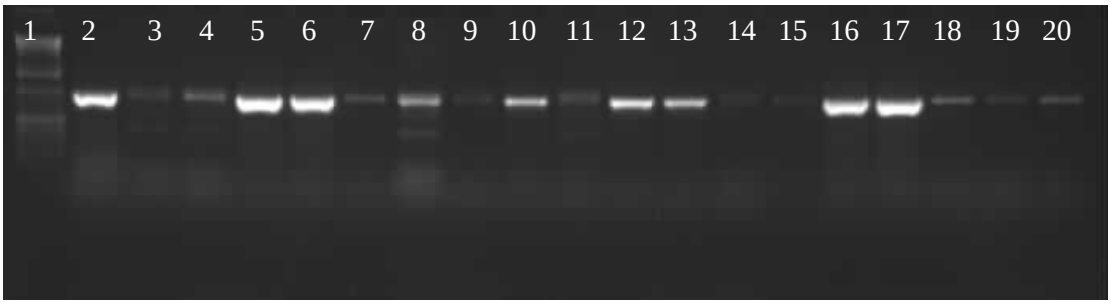


Fig: 4.5 Agarose gel electrophoresis of the PCR products from the progeny P6 (P6-13 to P3-24) and P7 (P7-1 to P7-6) of the R_3T_1 lines.

Lane1: 1 Kb DNA ladder

Lane 2-13: P6-13; P6-14; P6-15; P6-16; P6-17; P6-18; P6-19; P6-20; P6-21; P6-22; P6-23; P6-24.

Lane 14: -ve Control, C-19

Lane 15-20: P7-1; P7-2; P7-3; P7-4; P7-5; P7-6

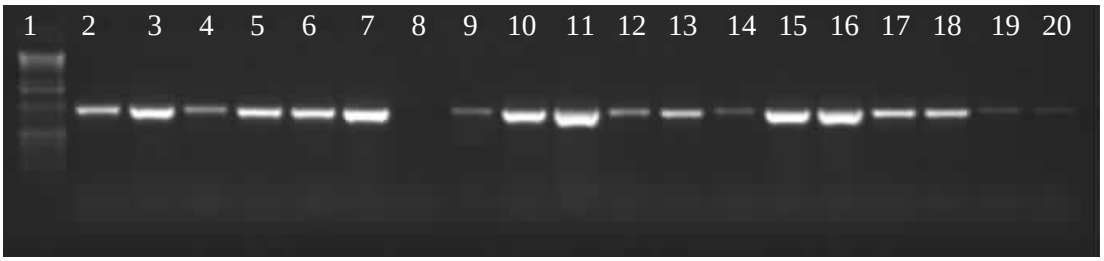


Fig: 4.6 Agarose gel electrophoresis of the PCR products from the progeny P7 (P7-7 to P7-24) of the R_3T_1 lines.

Lane1: 1 Kb DNA ladder

Lane 2-7: P7-7; P7-8; P7-9: P7-10; P7-11; P7-12.

Lane 8: -ve Control, C-20

Lane 9-20: P7-13; P7-14; P7-15; P7-16; P7-17; P7-18; P7-19; P7-20; P7-21; P7-22; P7-23; P7-24.

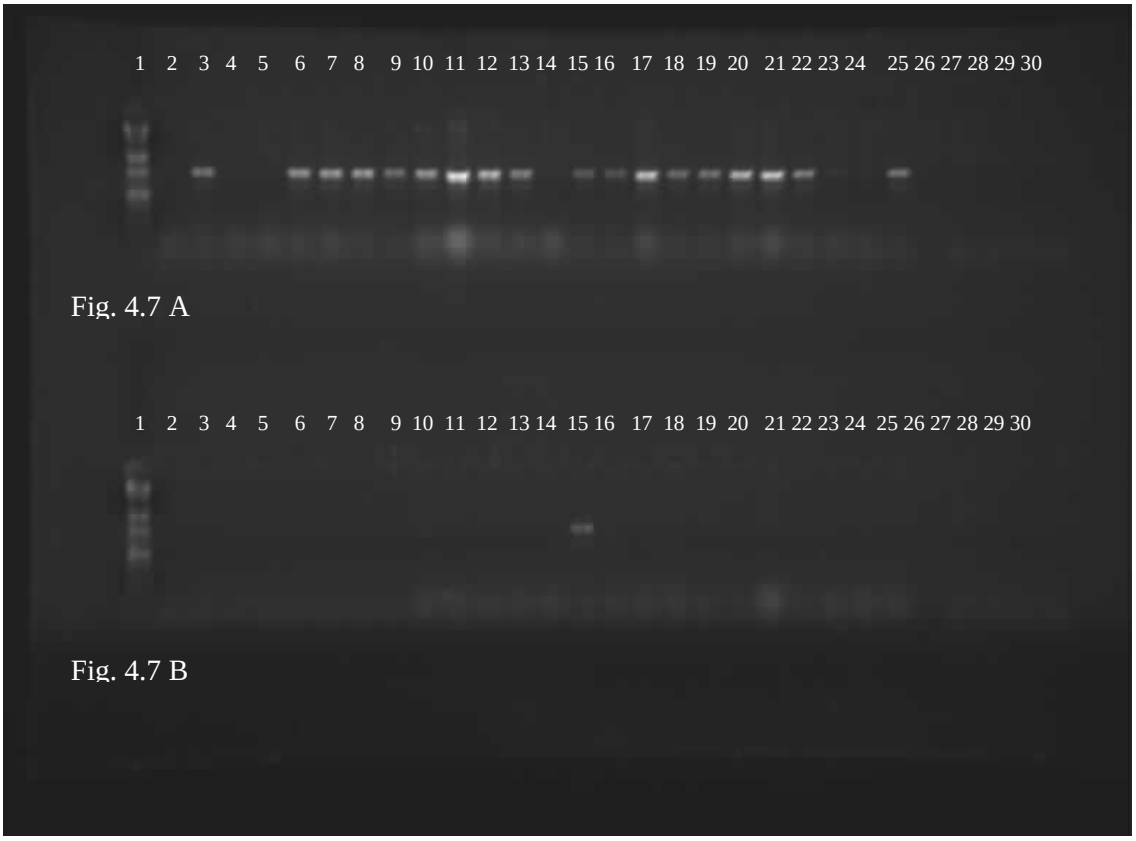


Fig 4.7 Agarose gel electrophoresis of the PCR products from the progeny P8 (P8-1 to P8-24) and P9 (P9-1 to P9-24) of the R_3T_1 lines.

4.7 A

Lane1: 1 Kb DNA ladder

Lane 2-25: P8-1; P8-2; P8-3; P8-4; P8-5; P8-6; P8-6; P8-7; P8-8; P8-9: P8-10; P8-11; P8-12; P8-13; P8-14; P8-15; P8-16; P8-17; P8-18; P8-19; P8-20; P8-21; P8-22; P8-23; P8-24.

Lane 26-27: -ve control, C-21; C-22.

4.7 B

Lane1: 1 Kb DNA ladder

Lane 2-14: P9-1; P9-2; P9-3; P9-4; P9-5; P9-6; P9-6; P9-7; P9-8; P9-9: P9-10; P9-11; P9-12; P9-13.

Lane 15: +ve control, plasmid DNA

Lane 16-25:; P9-14; P9-15; P9-16; P9-17; P9-18; P9-19; P9-20; P9-21; P9-22; P9-23; P9-24.

Lane 26-27: C-21; C-24.

Table 4.1: Percentage of the plants found positive for the transgene *Le Rab11a* in the progeny P1 to P9.

Progeny of T_1 R_3	Total plants tested	Positive	Percentage
P1	24	22	91.66%
P2	24	18	75%
P3	24	18	75%
P4	24	24	100%
P5	24	20	83.33%
P6	24	18	75%
P7	24	21	87.50%
P8	24	17	70.83%
P9	24	0	0

The PCR analysis of the progeny of R_3T_1 lines *viz*. P1 to P9 (24 plants for each individual line e.g. for P1 plants from P1-1 to P1-24) was done to check the Mendelian segregation ratio and to test the homozyogosity. Out of 9 lines 8 were found to be homozygous for the gene. Progeny of line P1, P2, P3, P4, P5, P6, P7 and P8 were found homozygous, whereas, in case of line P9, no transgene was found. In the PCR results the band of expected size was observed which is similar to Lu *et al*., (2001). The table 4.1 shows the summary of the PCR results. Line number P4 (100%) and P1 (91.66%) were selected for the further analysis in the present study.

4.1.2 Conclusion:

The PCR results of the progeny confirmed the homozygosity of the transgene. The transgenic *LeRab11a* Antisense line P4 was selected for the further studies to carry out the phenotypic characterization and textural analysis. The fruits at different developmental stages were tagged and harvested for the RNA Extraction for obtaining the gene expression profile (Chapter 6) of the different genes in *LeRab11* clade in the wild type and transgenic line.

4.2 Phenotypic characterization of the wild type and *LeRab11a* Antisense lines.

The phenotypic characterization of the wild type and transgenic *LeRab11a* antisense plants growing in the glasshouse was done. There was a remarkable difference in the growth and development of the branches, leaves, inflorescence and fruits.

Fig. 4.8 shows the different growth and branching pattern of wild type and antisense transgenic lines. Four pots of wild type and transgenic plant are shown. The four pots on the left shows the normal leaf and branching pattern in wild type plants AC++ whereas four pots on the right side clearly show the differential profuse branching pattern. The branching pattern of transgenic plants is similar as reported by Lu *et al*., (2001)



Wild Type Plants Transgenic Plants

Fig. 4.8 Growth pattern of wild type and *LeRab11a* Antisense Transgenic plants
The plants of wild type plants (four pots on the left side) show normal branching and leaf pattern as compared to the distinct morphological variations in branching and leaf shape of the antisense transgenic plants (four pots on the right side).

Fig.4.9 shows the normal branches and inflorescence with normal flowers of wild type tomato plants as compared to the transgenic plants. In fig.4.9 from A1 to A4 all show normal branches and leaves whereas in case of B1 and B2 the characteristic feature of the transgenic plants are shown, where instead of terminating into cluster of flowers one of the segment reverts to vegetative state and continues to grow into branches with flowers. This characteristic feature was also reported by Lu *et al*., (2001) and was found in majority of the transgenic plants. The meristem reversal is discussed in chapter 7.



Fig. 4.9 Branches and inflorescence of wild type and antisense transgenic lines

A1, A2, A3: Wild type normal branch and normal inflorescence; A1, A2 and A3 all show similar regular inflorescence with normal flowers in wild type AC++ plants. B1: shows the branch of a transgenic plant which continues to grow instead of terminating into the flowering truss bearing fruits. The lower end of the branch instead grows and forms another branch and bears inflorescence and again forming apical buds. 1-Arrow shows the point where the growth continues to form branch. 2-Arrow shows the inflorescence bearing flowers.



Fig.4.9 Branches and inflorescence of wild type and antisense transgenic lines

A4 shows the normal branch in wild type; B2 shows the branch of a transgenic plant which continues to grow instead of terminating into the flowering truss bearing fruits into regular vegetative branch. 1-Arrow shows the point where the vegetative growth continues to form new branch.

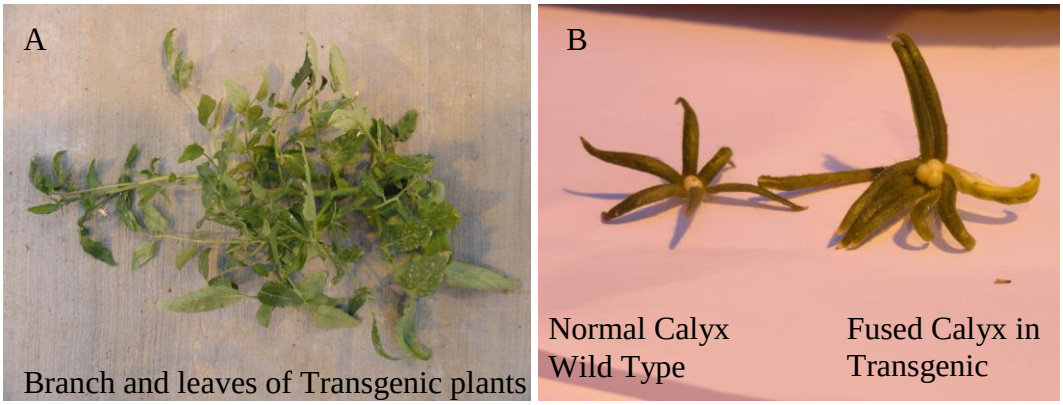


Fig.4.10 (A) Developmental abnormalities found in the transgenic leaves, (B) Comparison of the calyx of wild type flower and transgenic flower. The flower of wild type (AC++) on the left shows the normal calyx with six sepals and the flower with 7 DAP fruit shows the abnormal fused calyx (right).

Fig. 4.10-A: Shows the developmental abnormalities characteristics of the antisense *LeRab11a* transgenic lines with profuse branching and rumpled leaves. Fig. 4.10-B: shows the fertilized flower of wild type (AC++) and transgenic of seven days post anthesis. Wild type has normal flower with normal calyx with six sepals whereas, in

case of transgenic it shows the fused and larger sepals with one showing yellow colour instead of green. Similar observations were reported by Lu *et al*., (2001)



Fig. 4.11 Wild type and *LeRab11a* antisense transgenic plants in the glasshouse. A shows tomato plants in the glasshouse. A-(L) the left row - normal growth habit of wild type AC++ plants; A- (R)- right row -Antisense transgenic plants. B1, B2 and B3 shows the growth habit of transgenic plants showing profuse branching.

Fig. 4.11 shows the differential growth habit that of normal wild type plant and transgenic plants showing profuse branching pattern similar to that reported by Lu *et al*., (2001).

For fruits two different experiments were conducted. The flowers were tagged at the stage at which the anthesis occurs. In one experiment the fruits were harvested at mature green stage and stored under controlled environment chambers whereas in other experiments the fruits were left on both wild type and transgenic plants and the observations were recorded at different stages of fruit ripening.

Fig. 4.12 shows the developmental series of the tomato fruit development at different stages. The size of immature green fruit (IMG) increases through to mature green fruit (MG). The colour starts to change from green to slightly orange colour at the breaker stage (B). The fruit turns red after B+5, and B+10 and attains optimal red colour and which then becomes full red ripe (RR) around B+20 as shown in the fig.4.12.



Fig. 4.12 The developmental series of the normal wild type tomato fruits AC++
From left: IMG: Immature green; MG: Mature green; B: Breaker, B+10: Breaker +10, RR: Red Ripe-Breaker +20.

Fig. 4.13 shows wild type AC++ tomato fruit at breaker stage. The mature green fruits starts changing its colour from green to orange indicative of process of initiation of the ripening of fruit and change of colours from green to orange, pinkish red and finally to red. The change in colour progresses from bottom to top of the fruit as shown in fig.4.13 B with arrow.



Fig. 4.13 Tomato fruit of wild type Ac++ showing the breaker stage. (A) Side view of tomato fruit at Breaker stage (B) Bottom view of tomato fruit at breaker stage. Arrow shows the pattern of change in colour which progresses from bottom to top side of the fruit at breaker stage. Where the colour starts changing from green to pinkish red and orange at bottom first and then progresses towards the top.

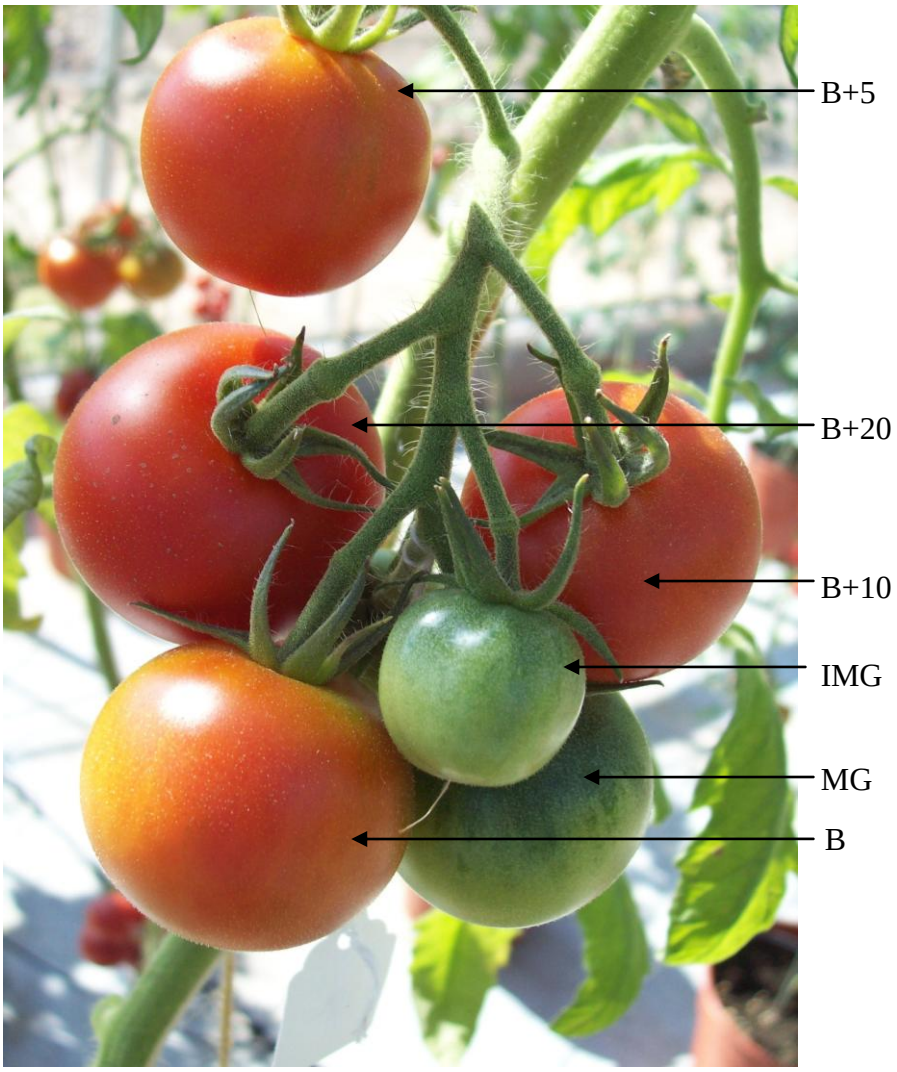


Fig. 4.14 Tomato fruits of antisense *LeRab11a* transgenic lines.
IMG: Immature green fruit; MG: mature green fruit; B: Breaker, B+5: Breaker + five days; B+10: Breaker plus 10 days and B+20: Breaker plus20 days red ripe fruit.

Fig. 4.14 shows the tomato fruits of antisense *LeRab11a* transgenic lines. The fig. shows tomato fruits at different developmental stages which were similar to the normal wild type tomato fruits developmental series (Fig. 4.12). In one cluster it shows the immature green fruit (IMG), mature green fruit (MG), breaker (B), breaker plus five days (B+5), breaker plus 10 days (B+10) and breaker plus 20 days (B+20) red ripe fruit. Thus indicating the transgenic tomato fruits are undergoing the normal ripening process stage-wise apart from the fact about the firmness of fruits at later developmental stages (chapter 5).

One of the characteristic of the *LeRab11a* antisense transgenic plants was different branching pattern with respect to the floral and vegetative meristem developing from the flower cluster and continues to grow into branches with leaflets, flowers and bearing fruits (Fig.4.15). As shown in fig. 4.15 A, B, C and D represent similar branching pattern in different transgenic lines grown in the glasshouse. A and its inset A1 shows that the branch continues to grow after the red ripe fruit at the end. Likewise, in case of fig.4.15-B, the young flowering branch with mature green fruits at the proximal region and fertilized flowers at distal region of the truss are shown which are at different developmental stages. The arrow and the circle indicate point of initiation of new developing vegetative branch with young leaves growing at the tip. In case of Fig.4.15-C and its inset C1 shows a much developed inflorescence with fruits at advanced stages of ripening including the red ripe, immature green and breaker. As can be seen from the fig. just after the fruit in the row (fruit at breaker stage) the branch out growth can be clearly marked which continues to grow at the end of the tip next to the last fruit indicated by circle and arrow. D and its inset D1 also show the same phenomena but the type of flowering cluster is different as compared to A, B and C transgenic plants. The yellow arrow and circle represent bifurcation of branch one into flowering and fruiting cluster and the other into a new branch with leaves. The sky blue arrow and circle show where the branch diverges to the point and continues its growth upwards. The red arrow and circle show the healthy young branch with young leaves and buds. Thus similar branching pattern was observed in the population of the transgenic plants grown in the glasshouse as was reported by Lu *et al*., (2001). This also confirms that the phenotype of the initial transgenic plant was hereditary and was transmitted to the next generations of transgenic plants. The probable cause of branching pattern is discussed in chapter 7.

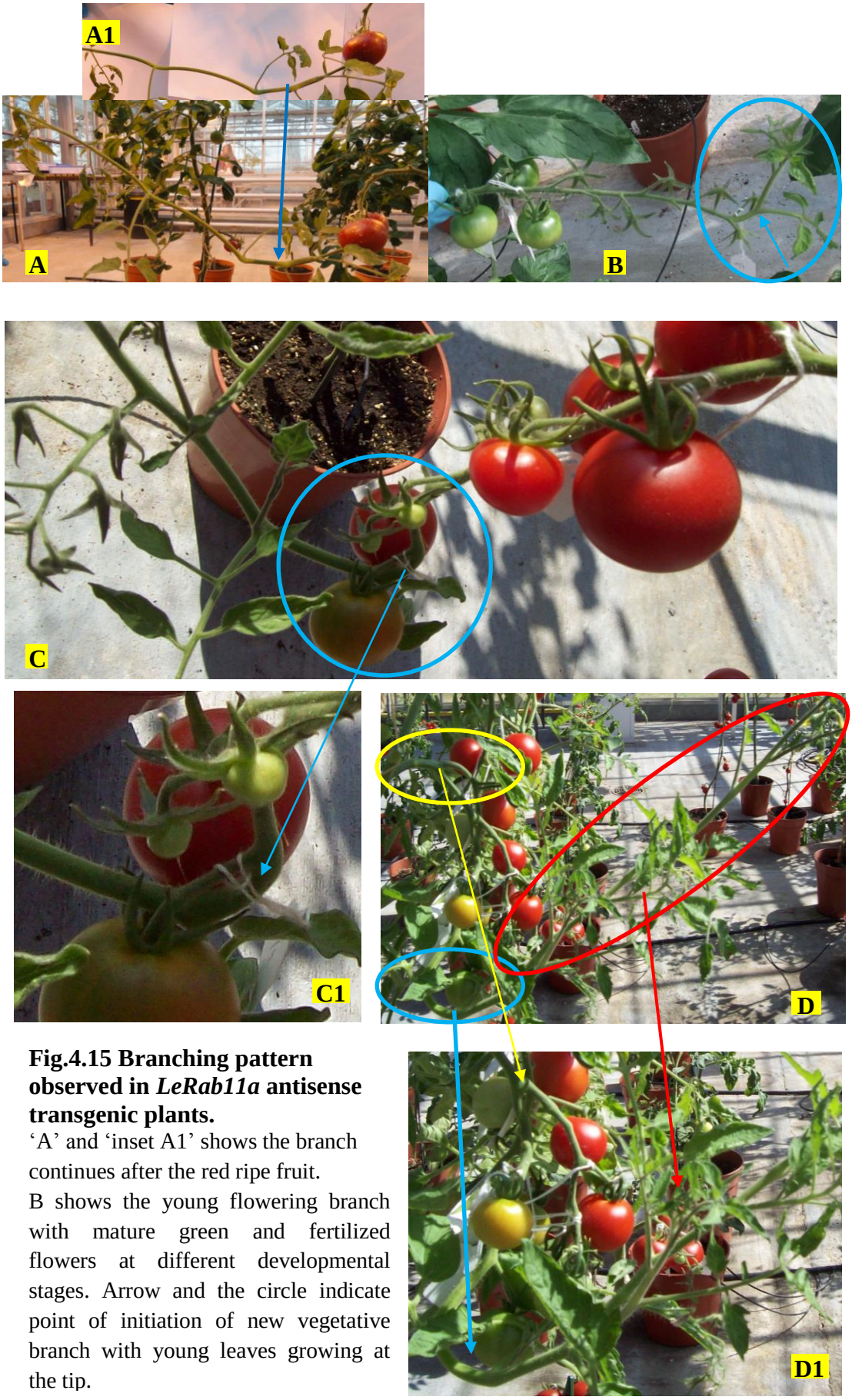


Fig.4.15 Branching pattern observed in *LeRab11a* antisense transgenic plants.

'A' and 'inset A1' shows the branch continues after the red ripe fruit.

B shows the young flowering branch with mature green and fertilized flowers at different developmental stages. Arrow and the circle indicate point of initiation of new vegetative branch with young leaves growing at the tip.

C and inset C1 shows a much developed and inflorescence with fruits at advanced stages of ripening immature green, mature green, breaker and red ripe with the young branch continues to grow at the end of tip next to the last fruit indicated by circle and arrow. D and inset D1 also show the same phenomena but the type of flowering cluster is different as compared to A, B and C. The arrow and circle represent the following: Yellow bifurcation of branch into new and one into flowering and fruiting cluster. Sky blue show where the branch diverges to the point. Red shows healthy young branch with leaf primordial and buds.

One of the other interesting phenotypic features of the transgenic plants was different types of flowering clusters or inflorescence or flowering trusses were observed. Unlike the uniform simple panicle inflorescence pattern found in wild type plants (AC++), different types of inflorescence bearing different flowering patterns were observed in the transgenic plants. Similar results were reported by Lu *et al*., (2001). In fig.4.16 from A1-A8 different patterns and clusters from different transgenic plants are shown, some times even different patterns were also observed in the same plant.



A1



A2



A3



A4

Fig. 4.16 Different types of inflorescence found in transgenic plants. Flowering truss bearing fruits of *LeRab11a* antisense transgenic plants. A1, A2, A3 and A4 all show different pattern of different transgenic plants grown in the glasshouse.



A5



A6



A7



A8

Fig.4.16 Different types of inflorescence/truss bearing fruits of *LeRab11a* antisense transgenic plants. Truss bearing fruits of *LeRab11a* antisense transgenic plants. A5, A6, A7, and A8 all show different patterns truss in different transgenic plants grown in the glasshouse.

The main and the striking feature of the antisense transgenic plants was the enhanced shelf life of transgenic tomatoes as compared to the control wild type. Fig.4.17.1 and Fig.4.17.2 show the differential pattern of wild type and transgenic fruits at later stage of ripening when left on vine or plant. In fig.4.17.1-A- The wild type tomato fruits

were tagged at mature green stage and were left on plants for equal number of days to that of transgenic fruits. After 95 days post MG stage as shown in fig.4.17.1 (A,B and C) the wild type tomato fruits lost shape, structure, turgidity, firmness and were infected by fungus. In B and C the transgenic tomato fruits still retained the shape and turgidity as compared to the wild type plants. Remarkably they did not loose much water (discussed in chapter 7) and were not infected by the fungus. They were firmly attached to the calyx instead of developing abscission zone and falling off the plants after detaching form the calyx.



A **B** **C**

Fruits of Wild Type Fruits of Transgenic plants left on branches



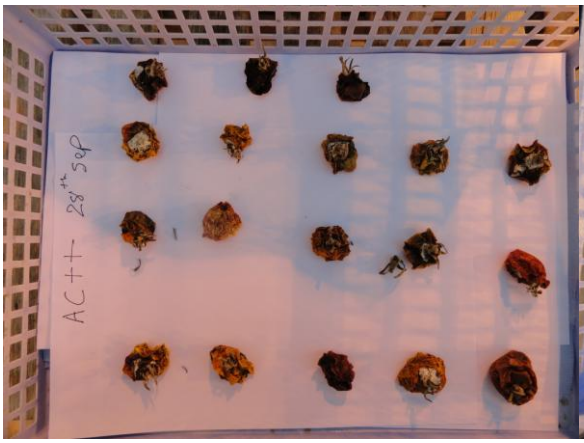
D1 **D2** **E** **F1** **F2**

Fruits of Wild Type Fruits of Transgenic plants left on branches

Fig.4.17 Differential pattern of wild type and transgenic fruits at late stage of ripening when left on vine/plant. A-Wild type tomato fruits left on plants; B & C transgenic tomato fruits (A, B and C 95 days after MG stage). D1 and D2 tomato fruit of wild type plants after 95 days of MG stage. E- Transgenic tomato fruits on vine after 100 days after MG stage. F1 the transgenic fruits left on the vine for 95days after MG stage. F2- transgenic tomato fruits harvested after 95 days post MG stage of GP4 transgenic line.

In fig.4.17 D1 and D2 are the normal wild type tomato fruits left on the plants for 95 days post MG stage. Both D1 and D2 exhibit loss of fruit integrity, water, shape, structure, turgidity, and severely infected by fungus and collapsed. E-shows still intact transgenic tomato fruits of GP4 transgenic line left on plants for 100 days after MG stage, the fruit did not loose shape, structure and integrity and was not infected by fungus. F1 and F2 show fruits of GP4 transgenic plants. In case of F1 the fruits did

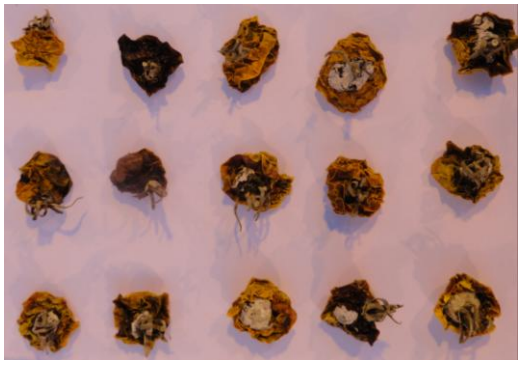
not loose their shape, structure and integrity (discussed in chapter 7) and were not infected by the fungus. They were firmly attached to the calyx. F2 shows the fruits harvested after 95 Days post MG stage retaining colour, turgor, shape and integrity.



A1-Fruits of wild type tomato AC++ Set-I



B1-Fruits of Transgenic tomato Set-I



A2-Fruits of Wild Type tomato AC++ Set-II



B2-Fruits of Transgenic tomato Set-II

Fig. 4.18 Differential pattern of wild type and transgenic fruits at late stage of ripening after harvesting and storing. Fruits after 110 days of harvesting post MG stage. A1 (Set-I) and (A2-SetII) infected and dried WT fruits. B1(Set-I) and B2 (Set-II) transgenic fruits with no infection.

Another experiment was conducted to observe the behaviour and softening patterns of wild type and transgenic tomato fruits after they are harvested and stored until they loose their shape, colour, size, integrity, turgidity and to check whether they are infected by fungus. Two different sets were used where the fruits from wild type plants and transgenic plants were harvested at mature green stage and were stored. As shown in fig. 4.18 in Set-I and Set-II both had similar results for wild type and transgenic fruits. Wherein the wild type tomato fruits (A1 & A2) completely lost the shape, colour, size, integrity, turgidity collapse, reduced to dry peels and were severely infected by the fungus. But in case of transgenic fruits (B1 & B2) in both

cases the fruits showed an extend shelf life as compared to wild type. They retained fair amount of frimness, shape, integrity, colour, did not collapse and were not infected by fungus as was observed when transgenic fruits were left on vine in other experiment. The calyx too was firmly attached to the transgenic fruits, and it was very difficult to take them off the fruits. In case of transgenic fruits the skin was shriveled but the fruit was still attached to the calyx and because of this there was no onset of any fungal infection in case of transgenic fruits even after such a long time of storage. The fruits of transgenic plants retained water and showed a pattern of delayed softening, whereas WT fruits got infected, which needs further investigation and a proper set up of either natural or artificial inoculation of the fungus on both wild type and transgenic fruits to see if there is a difference and what could be the possible mechanism for either tolerance or resistance to fungus. The leaves, flowers, calyx, inflorescence and other developmental abnormalities of transgenic plants and fruits showed all the phenotypes described by Lu *et al.*, (2001) which are characteristic of the *LeRab11a* antisense line and also showed delayed pattern of fruit softening.

5. Textural analysis of Tomato fruits

5.1 Introduction

Fruit softening is a complex trait; in case of fleshy fruits the fruits undergo several physiological changes including change in colour, flavour and texture. Texture depends on many structural qualities such as the proportions of flesh to seeds, size of cells, shape of cells, strength of cell wall, and strength of connections between cells. The firmness of tomato is one of the most important factors in achieving tomatoes with good texture and shelf life during storage and transport. Tomatoes may be too firm, or not firm enough. The structure of the tomato affects firmness. The presence of small cells just under the cuticle (in the skin) of the tomato seems to result in a tougher skin and a firmer overall texture. The strength of cell walls and connections between cells in the pericarp or fleshy part of the tomato is also important, the stronger these connections and cell walls results in the firmer tomato fruit. It is the fleshy bit of the tomato (pericarp) that is responsible for this texture. If the cell walls are quite strong but the connections between them are weak then tomato will have a mealy texture. Juiciness is the amount of liquid released by the tomato during ripening and post ripening stages and during storage. The more water content the cells have the juicier the flesh. The weaker cell walls degrade easily and more readily release their liquid content by respiration and results in the degradation of the texture and shelf life of tomato fruit under storage conditions.

The texture of tomato is dependent on the physical properties of the tomato. The cell wall strength and the subsequent changes during different developmental stages of ripening affect the tomato fruit firmness. The ripening of tomato in terms of softening at later stages and alteration in the texture is because of the breakdown or digestion of the cell walls by different enzymes. PG, PE, cellulase etc., are important enzymes which break down the cell wall.

The most important parameters indicating the tomato fruit quality are its firmness, colour and shelf life (Polderik *et al*., 1993). The degree of firmness of fruit indicates its maturity, freshness and acceptability (Burton, 1982, Choi *et al*., 1995).

PG activity is absent in green tomato fruits but is expressed during the ripening (Tucker and Grierson, 1982). There are few direct measurements for assessing the changes in the texture of the tissues in the specific regions of the fruit. The tissue softening of fruits is a more precise way of assessing the structural changes occurring during the ripening process. Tomato fruits have relatively short shelf life. The losses during the storage and transportation make the ripening and fruit softening of tomato of great economic importance. To be able to modulate the texture and enhance the shelf life of tomato fruits without altering its texture, colour, and taste is essential. There is positive correlation between the ripening of tomatoes and their physical properties such as colour and fruit firmness. The loss in firmness is associated with the softening of the fruits and changes in the colour of the skin which are usually affected by various factors such as temperature, maturity stage, atmosphere, relative humidity, storage time and the genetic makeup of the plants (Wu and Abbott, 2002).

A decline in the fruit firmness is because of the dissolution of the middle lamella, resulting in the reduction of the intercellular adhesion, de-polymerization and solubilization of hemicellulose and pectic cell wall polysaccharides (Brummell and Harpster, 2001)

5.2 Textural analysis of wild type and transgenic tomato fruits.

The textural analysis of the wild type and transgenic fruits was done at different developmental stages. The firmness of the fruits was measured by compression and penetration test. The fruits were tagged and harvested at different developmental stages at mature green (MG), Breaker (B), B+5, B+10, B+15, B+20. TAXT2 stable microsystem was used for the textural analysis. For each test 6 fruits were used as replicates to perform the compression and penetration tests. The fruits of equal developmental stage, size and weights were taken. The data was analyzed by two tailed student t-Test.

5.2.1 Compression test

Compression test is a non destructive method for measuring the fruit firmness. For compression test readings were recorded in two different ways one reading for polar and three equatorial reading. For polar readings the probe was set to compress the tomato fruits from the top where the calyx is attached, the calyx was removed before

performing the compression tests. For compression test, the test speed was kept at 0.5 mm/sec and the test distance was kept as 4 mm. The force was measured in ‘g’.

As shown in the fig. 5.1, the first peak of the curve shows the polar reading while compressing the tomato from the top side at the point where calyx is attached to the fruits.

The next three curves in the graph shows the equatorial readings which were taken from the equatorial side of the tomato. Each reading was recorded around the different sides of the equatorial plane of the fruits.

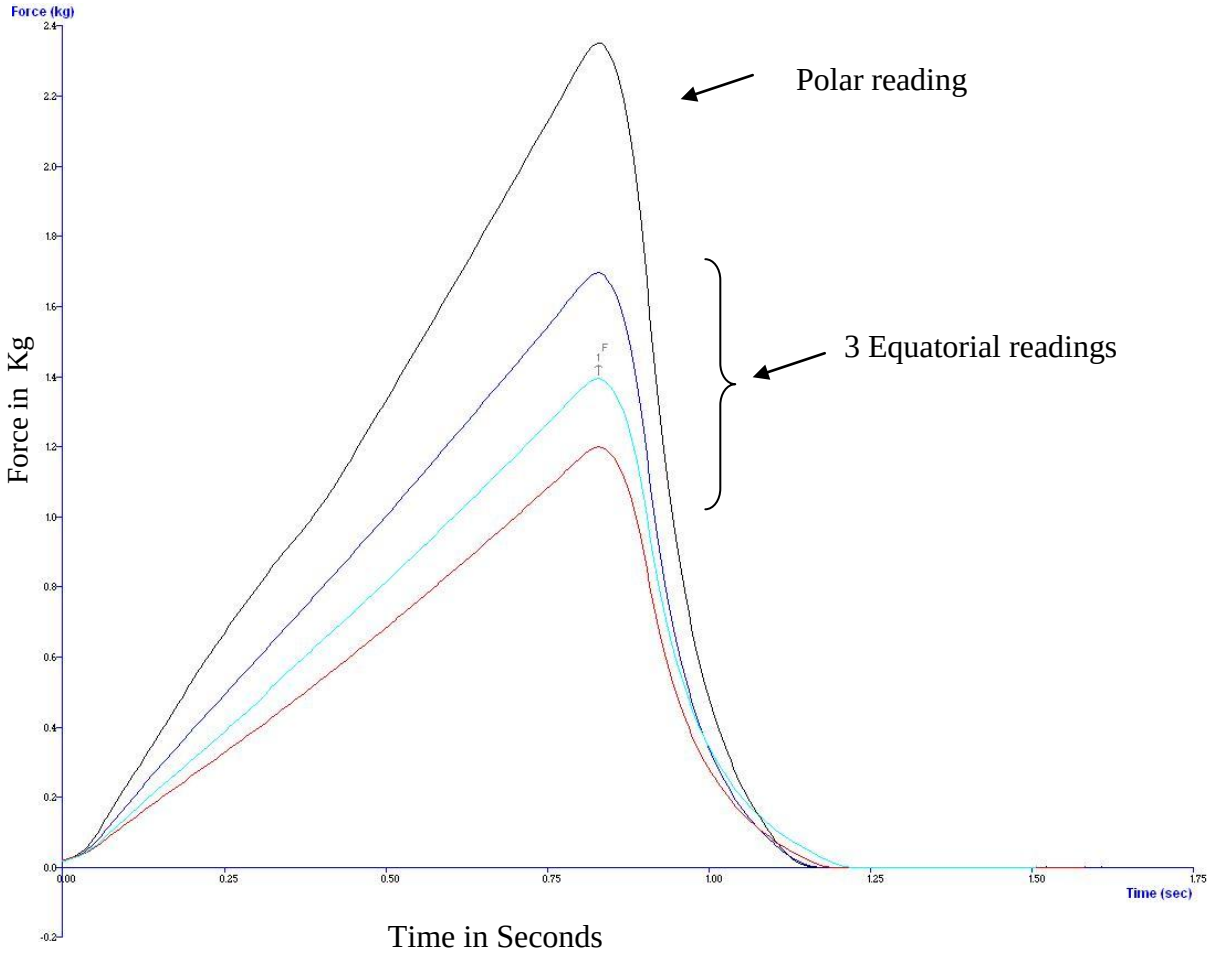


Fig.5.1 A typical graph showing compression of whole tomato fruit.
First curve shows the polar compression, the other three curves below show the three equatorial compression readings. The graph shows the force required and time in seconds adjusted for the probe to compress the fruit from top to bottom.

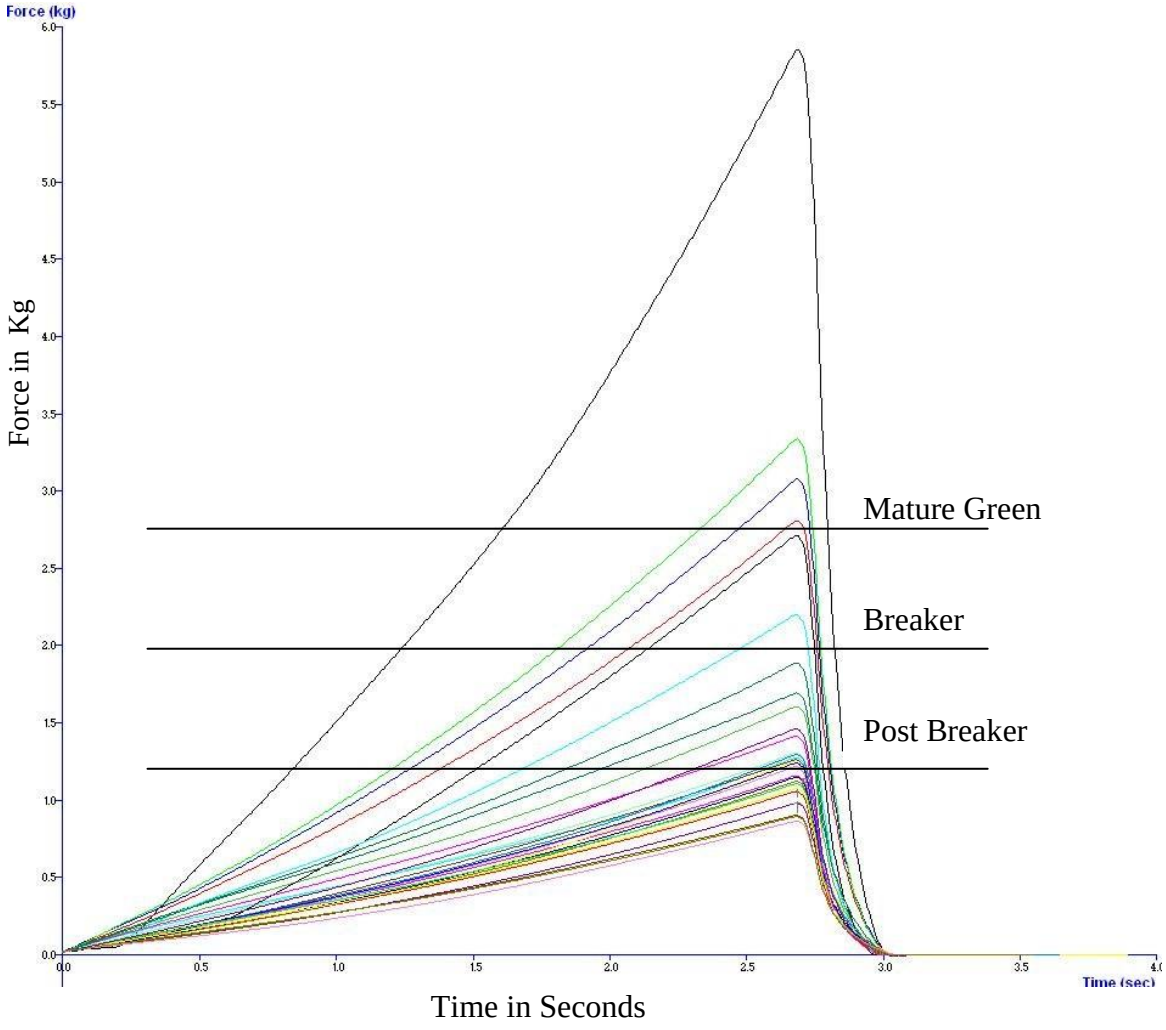


Fig.5.2 Typical graph of compression test of whole tomato fruits at different developmental stages. Total four readings were recorded (1 polar and 3 equatorial) for each fruit. The black lines across the graph shows differences in the force required to compress mature green, breaker and red ripe fruit. The graph shows the force required and time in seconds adjusted for the probe to compress the fruit.

During the compression test of tomato fruits at different stages it was found that as the fruit reaches from mature green stage to post breaker stages the force required to compress the fruit was decreasing. As shown in the fig. 5.2 first peak shows one polar and 3 equatorial reading at mature green stage. More force is required to compress the fruit which gives higher readings, and likewise the force required to compress the fruits at breaker and post breaker stages goes down as the fruit starts to soften, this graph shows the compression test done on the wild type tomato fruits. Thus the compression test is a good indicator of fruit firmness and is a non destructive method of measurement of fruit firmness.

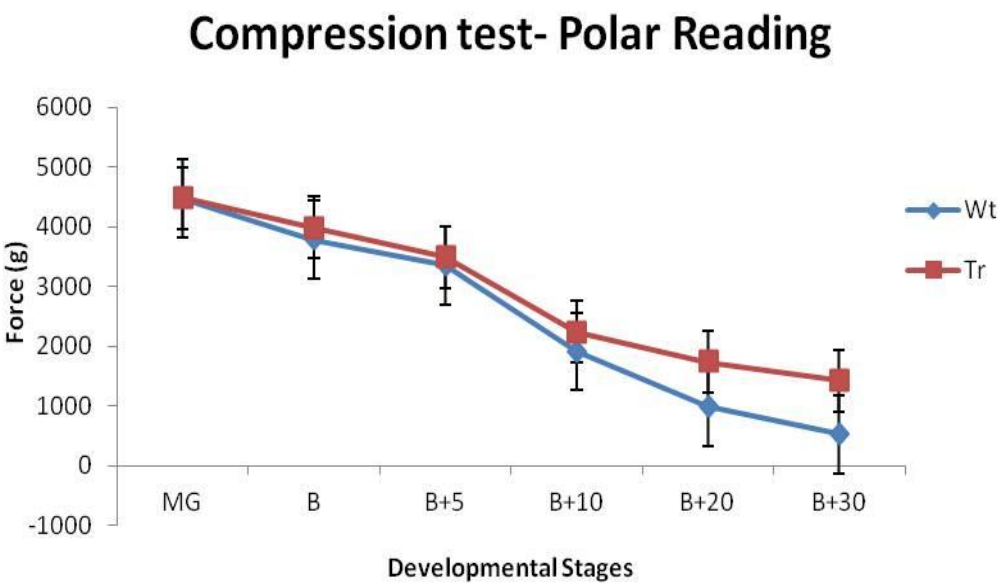


Fig.5.3 Compression test of wild type and transgenic fruits based on polar readings at different developmental stages. One polar reading was recorded for each fruit and for 6 fruits at each developmental stage. MG-mature green; B-breaker; B+5-breaker + 5 days; B+10-breaker + 10 days; B+20-breaker + 20 days; B+30-breaker + 30 days. Wt-wild type fruits (AC++); Tr- *LeRab11a* transgenic fruits.

For the comparison between the wild type and transgenic *LeRab11a* antisense lines the polar reading from 6 fruits were pooled and the average was used to compute the fruit firmness index based on the polar readings. As shown in the Fig.5.3 there is no significant differences at the initial mature green stage of the development of fruits during the ripening in tomato fruits in either transgenic or wild type. But there is remarkable difference between the WT and transgenic fruits at post breaker stages.

After B+20 there is a significant difference in the fruits as the transgenic fruits retain the firmness where as the wild type fruits start to soften. After breaker B+30 stage it was very difficult to do a compression test on the wild type fruits. Likewise in case of the compression test based on the equatorial readings the same results were obtained as shown in the Fig.5.4. The three readings from each fruit were pooled and the average was used for calculation. There was a remarkable difference from the compression test based on the polar reading because while taking the polar readings the fruit is compressed from the top, thus the columella gives a fair resistance toward

the polar compression. But in case of the readings taken from the equatorial plane of the fruits, the force required is relative less as seen in fig.5.4. At the post breaker stages after 25 to 30 day the wild type fruits soften gradually and collapses while compressing the fruit from equatorial plane, but in case of the transgenic fruit it still retained the firmness.

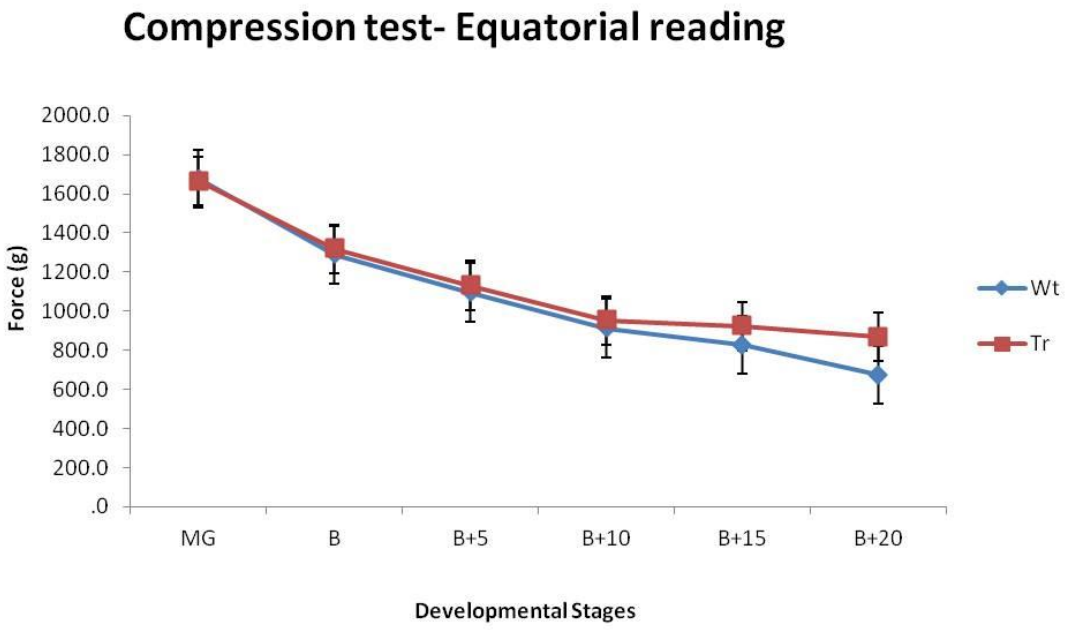


Fig.5.4 Results of the Compression test between wild type and transgenic fruits based on equatorial readings. Three equatorial reading were recorded for each fruit and for 6 fruits at each developmental stage. MG-mature green; B-breaker; B+5-breaker + 5 days; B+10-breaker + 10 days; B+15-breaker + 15 days B+20-breaker + 20 days; Wt-wild type fruits (AC++); Tr- *LeRab11a* transgenic fruits

5.2.1 Penetration test

In the penetration test the depth of penetration was measured under a constant load. The probe was made to penetrate into the fruits and the force necessary to achieve a certain penetration depth or the depth of penetration in a specified time, under defined conditions, was measured and used as an index of firmness for measuring the textural property of the fruits. The TA.XT2 analyzer was used to perform the test using BS757 0.5 diameter Delrin probe (2mm blunt probe) under standard settings. The pre test speed was kept at 0.5 mm/sec, and the distance for penetration was kept at 10 mm.

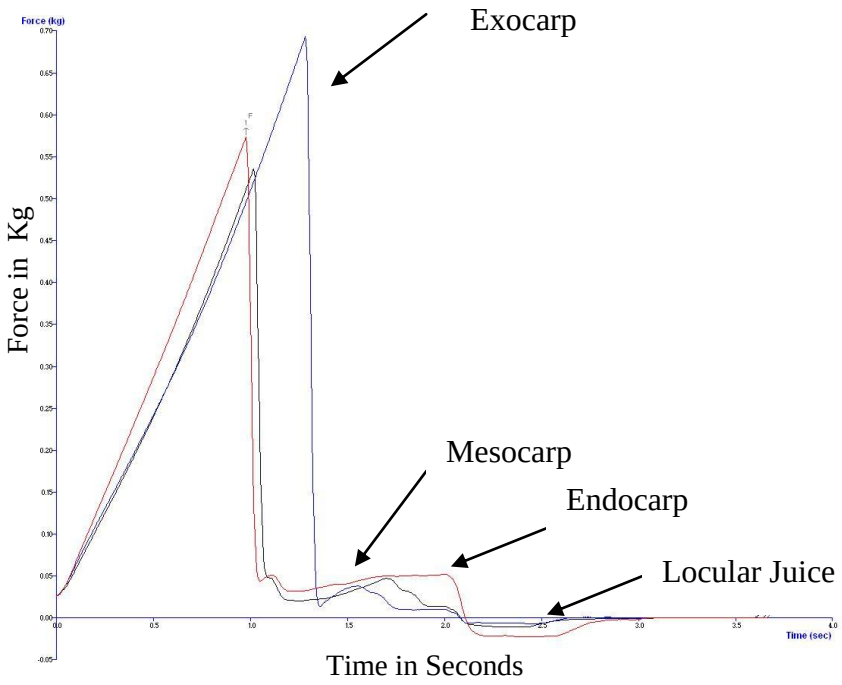


Fig.5.5. A typical graph showing the penetration test of whole tomato fruit. Equatorial plane of whole tomato fruit was used for penetration test. Three readings were taken for each fruit. The graph shows the first peak where the probe pierces the tomato skin/peel (exocarp) and progresses through the mesocarp and endocarp of fruits and finally reaches the inner side of tomato fruit where locular juice and seeds are present and then stops.

The penetration test was performed on the fruits on the equatorial region of the fruits. Six fruits from each stage were taken for the test and for each fruit three reading were taken from the equatorial plane of the fruits. The average of these readings was used for calculation. In the fig.5.5 first curve shows the first stage at which the probe penetrates at the equatorial side of the tomato fruit penetrating the exocarp. The other lower peak indicates when the probe enters the mesocarp and endocarp and then is retrieved back to the normal position. The other experiment was conducted on the 2 cm slices cut form the tomato fruits as shown in the fig. 5.6. It was easy at the beginning when the fruit was at mature green stage and firm but at post breaker stages it was nearly impossible to measure the force as there were so many variations in the readings. The other experiment was done on the half fruit of tomato after taking out the juice and seeds. As shown in fig.5.7 the first low peak shows the probe penetrating in the flesh of the fruit from the inside and the higher peak shows when the probe penetrates the skin of the fruit. Similar to the 2 cm cut slices it was only possible during the mature green stages in this experiment with fruits cut into half. So only the data obtained from the penetration test of the whole fruit was used for the analysis to measure and establish a fruit firmness index between wild type and transgenic fruits.

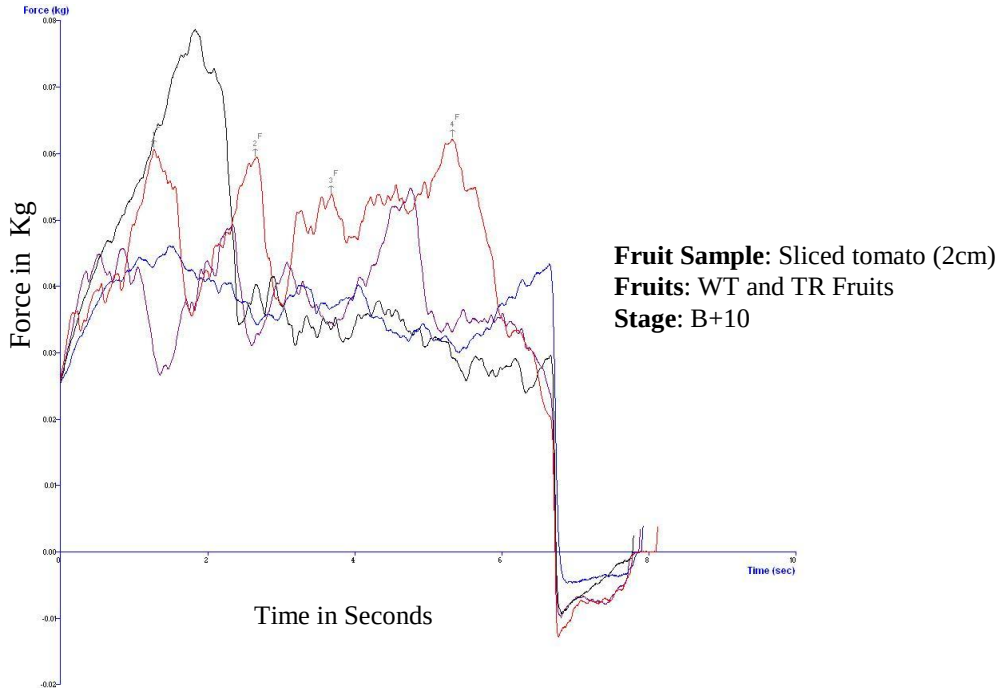


Fig.5.6 Penetration graph showing the penetration of the probe into the 2 cm thin sliced tomato. Penetration readings were recorded on sliced tomato (2cm). Two types of readings were recorded from inside out and outside in of the slice. WT-wild type fruits (AC++); TR- *LeRab11a* transgenic fruits B+10-breaker + 10 days;

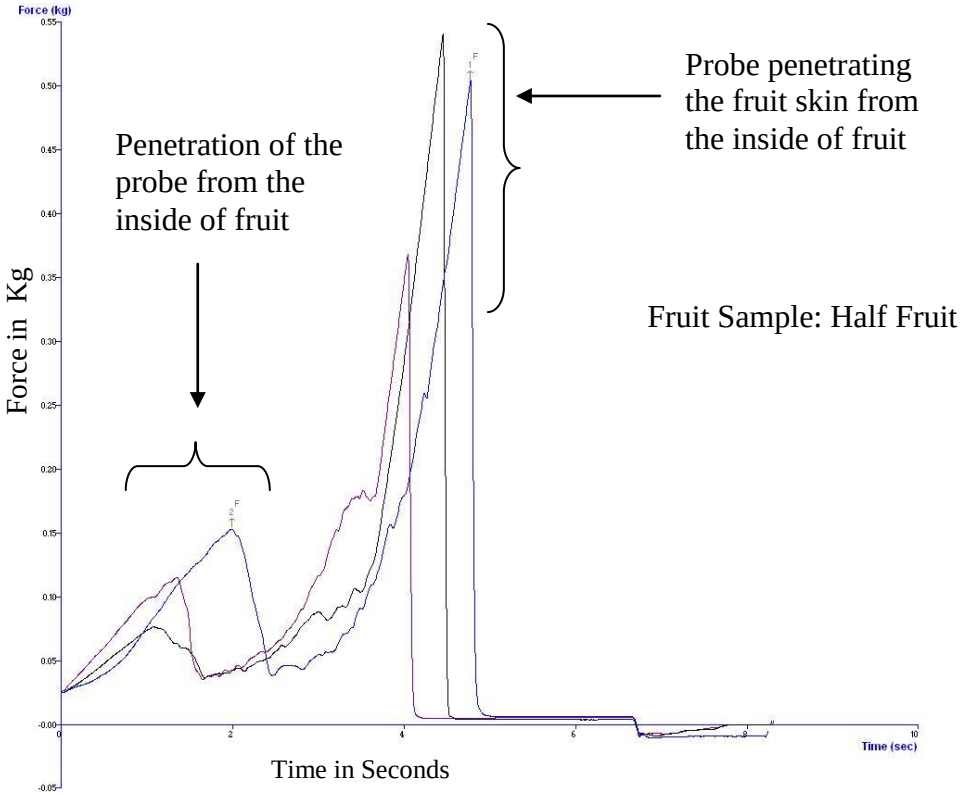


Fig. 5.7 Penetration test on the half tomato fruit. Graph shows the penetration of the probe into the half fruit. Whole tomato fruit was cut into half from the point where calyx is attached. Lower peaks at the beginning shows probe entering the flesh and the higher peaks show when the probe penetrates into the skin/cuticle of tomato

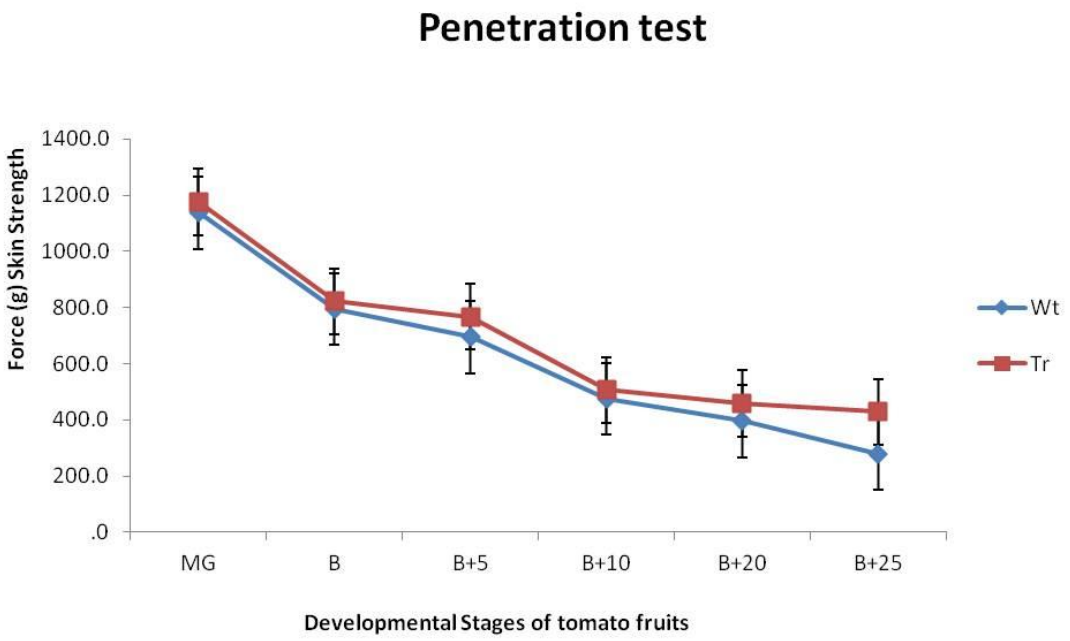


Fig. 5.8 Results of Penetration test between wild type and transgenic fruits. Three equatorial penetration readings were recorded for each fruit and for 6 fruits at each developmental stage. MG-mature green; B-breaker; B+5-breaker + 5 days; B+10-breaker + 10 days; B+20-breaker + 20 days; B+25-breaker + 25 days; Wt-wild type fruits (AC++); Tr-*LeRab11a* transgenic fruits

As shown in the Fig.5.8 there is no significant differences at the initial mature green stage of the development of fruits during the ripening either in transgenic or wild type fruits. But there is remarkable difference between the wild type and transgenic fruits at post breaker stages or post red ripe stage. As the fruit ripens it starts to soften gradually at post breaker stages. But in case of transgenic fruits they retain the turgor and firmness, it was very difficult to conduct penetration tests on wild type fruits even at B+25 and post B+25 stages. But the difference starts to appear after B+20, where there is a slow and delayed pattern of softening of fruits in case of *LeRab11a* antisense transgenic line. Beyond the B+30 stage there is a significant difference in the fruits as the transgenic fruits retain the firmness as observed in compression test where as the wild type fruits start to loose their turgidity and were more prone and susceptible to the fungal infections. These results confirm that there is a marked difference in the fruit firmness of wild type and transgenic fruits post breaker stages. The results of the textural analysis were similar to those reported by Lu *et al*., (2001).

6.1 Introduction

The quantitative real time RT-PCR is a reliable technique to find the gene expression and to generate the expression profile. The two methods used for qRT-PCR are either using the SYBR green or Taq-man Probes for more specific genes. For the relative gene expression profiling the most important fact is to isolate high quality RNA and synthesis of first strand cDNA to be used for real time PCR. SYBR green chemistry involves the ability of SYBR green to bind with the double stranded DNA. When the SYBR green binds to the double strand of the DNA it gives fluorescence which can then me measured by the machine at specific wavelength. As the DNA is synthesized during the PCR reaction the amount of double stranded DNA produced when bound to SYBR green gives a fair indication of the amount of DNA being produced in the reaction and reflected in terms of the fluorescence. To ensure the fidelity and precision of the qRT-PCR reaction the primer designing is of utmost important. To ensure that the final product of the reaction is the actual gene of interest, a melt curve analysis was done. The Roche Lightcycler 480 generates a melt curve at the end of reaction and can be checked by 'Tm' calling application in the software. If there is only one peak then the primer is working fine and there is no primer dimmer formation. The values obtained after the reaction is over gives the Cp or Ct values, which are used for further analysis and normalization in order to get the actual gene expression profile. The results of qRT-PCR were analyzed according the formula by Perikles (2003).

6.2 Sample preparation

The fruits from the wild type and transgenic *Le Rab11a* antisense lines were tagged and the fruits were collected at different developmental stages *viz.*,7DPA, 15DPA, 30DPA, mature green (MG), Breaker (B), B+5, B+10, B+20. The fruits were harvested, the seeds were removed, RNA was isolated and checked for quality on Nanodrop spectrophotometer. The RT-PCR was done to generate the first strand cDNA. For every reaction three biological replicates were used along with the no transcript (NTC) and no reverse transcript (NRTC) control.

6.3 Designing primers for qRT-PCR

The primers were designed for the quantitative real time PCR for the selected *Le Rab11* GTPase using PRIMER-3 software from the SGN website. While designing the primers the following parameters were used as standards for designing the effective primers for qRT-PCR.

1. T_m of primers: 58-60° C
2. GC%: 30-80
3. Primer length: 18-22 bp
4. Amplicon length: 50-150 bp

The most appropriate primer pairs for the genes satisfying the above criteria were selected. To check the primers for specific genes the forward and reverse primers sequences were screened using the BLAST search in the TIGR DFCI database. After exhaustive blast searches and screening the best primer sequence with the 100 percent hits was selected (Table 6.1). Likewise primers were designed for the house keeping genes (Table 6.2). The primers were then checked by normal RT-PCR on the pooled cDNA of all the developmental stages. As shown in the fig.6.1 the primers for the genes TC198194, TC207026 and TC207551 were designed again to carry on with the qRT-PCR. The layout of the experiment is shown in the Table 6.4

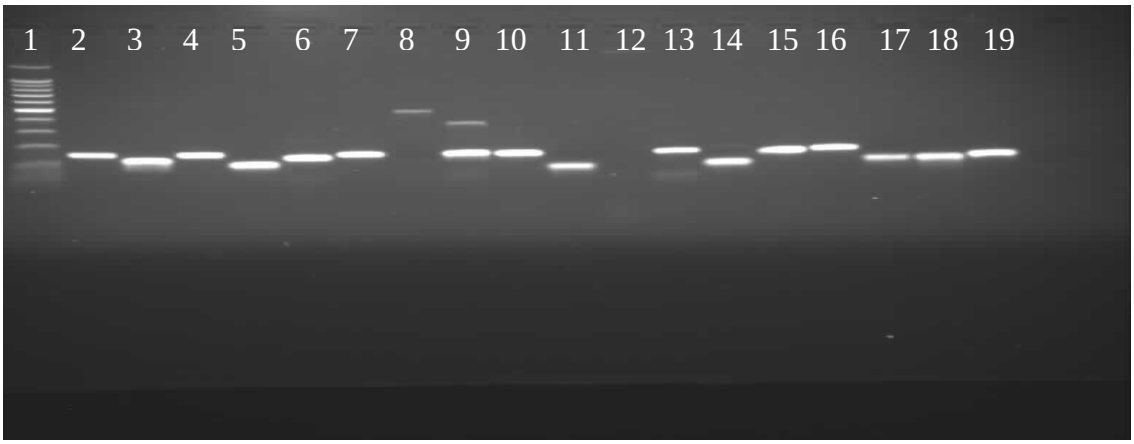


Fig.6.1 Results of RT-PCR of selected LeRab11 GTPase genes
Lane1: Marker
Lane 2-19: TC195734 ; TC193549; TC212461; TC192047; TC199565; TC195610; **TC198194**; **TC207026**; TC207267; TC204406; **TC207551**; **TC208438**; TC208692; TC212051; TC201753; TC211111; TC202127; TC211213. For lane 8, 9, 12 and 13 primers were designed again.

Table 6.1 Primer list for LeRab11 Class for qRT- PCR

Genes	Name of Primers	Primer Sequences
TC195734	TC195734fwd	TTCTTGAGACATCGGCATTG
	TC195734rvr	GGTTGTACCCTGACCAGGAA
TC193549	TC193549fwd	GAGCTTCCCCTTGAGTTCCT
	TC193549rvr	ATCAAAATTCACCCCATCCA
TC212461	TC212461fwd	TGTTGGAGATTCAGCTGTGG
	TC212461rvr	TCCCATATTTGAGCCTTGATG
TC192047	TC192047fwd	GACTGAGGACAACAGCAGCA
	TC192047rvr	CAATTGCAACCCGAGAAAAT
TC199565	TC199565fwd	AAAAGTTTGGGTGCTGTTCG
	TC199565rvr	ATCGCGATACCCAAAGTGAG
TC195610	TC195610fwd	CAAGGCCTTTGCTGAAAGAG
	TC195610rvr	ATCATCCCCAACTTCGAGTG
TC198194	TC198194fwd	TGCTTTTGGTGCTCTTGTTG
	TC198194rvr	TTTCCCACCAGTATCCTTGC
TC207026	TC207026fwd	ATGTTCTGAGGTGGCTACGG
	TC207026rvr	ATGACAGCCCTTCCTTCTCA
TC207267	TC207267fwd	AGAATGGAAACGGAAACGTG
	TC207267rvr	CGGTGCACCTAGAACAGGAT
TC204406	TC204406fwd	TTGTTGTTGGTGCAGAGGAG
	TC204406rvr	AACAGCAAGCGAGGAATCAT
TC207551	TC207551fwd	TTTGATCACATCGTGGGAGA
	TC207551rvr	CCTTGCTTCTTTTGCAGACC
TC208438	TC208438fwd	CCACCGAGGATGCTAAAGAA
	TC208438rvr	CGGCGAGATTCTTCTTGTTC
TC208692	TC208692fwd	TTCCACCACTCCTCATCTCC
	TC208692rvr	CGGCTACAGAAGGAAACAGC
TC212051	TC212051fwd	GAAACATCGGCTCTCGACTC
	TC212051rvr	GGCTAACGCGATTGACAGAT
TC201753	TC201753fwd	TAACCTTCCGCCCCTTTATT
	TC201753rvr	TCCCCAATCACCACAATCTT
TC211111	TC211111fwd	GCGTTGTTAGCAGGAAAAGC
	TC211111rvr	CATCCTTGCCTCCAACATTT
TC202127	TC202127 fwd	GTTGGAGGCAAGGATGATGT
	TC202127rvr	AGAATCTCTTCCGCGGTGTA
TC211213	TC211213fwd	GCAGTGGGTGCCTTACTTGT
	TC211213rvr	CGATGTTTTGGTCTGTGTGG

Table 6.2 List of Primers for house keeping genes

Genes	Primer Name	Primer Sequences
ACTIN	LeActFwd	TGG TGA TGA TGC TCC TCG AG
	LeActRvr	GTG CCT CAG TCA GGA GAA CA
GAPDH	GAPDHfwd	CTGCTCTCTCAGTAGCCAACAC
	GAPDHRvr	CTTCCTCCAATAGCAGAGGTTT
PGK	PGKFwd	CTTCCTCCTTAAAACTCCTCTCC
	PGKRvr	CTAAGGTCTCCAACGCTCTTCT
UBI	UBIFwd	GGACGGACGTACTCTAGCTGAT
	UBIRvr	AGCTTTCGACCTCAAGGGTA
EFT	EftFwd	ACC TTT GCT GAA TAC CCT CCA TTG
	EftRvr	CAC ACT TCA CTT CCC CTT CTT CTG

6.3 Primer Validation of Rab11 GTPase

6.3.1 BLAST Report of Individual Primer BLAST in TIGR Database

All the possible primer pairs obtained from the Primer3 software were used as query to run the BLAST search and tested in TIGR DFCI TGI Database.

The complete set of blast results of all the primers in all combination can be found in the additional files (Rab GTPase Primer Validation and Blast Results).

The main supplemental files for primer designing and validation are:

1. Primer Picking Results 'Blast Hits of All the Primer pairs for *LeRab11* genes in TIGR DB.txt'
2. Primer Picking Results from Primer 3 software and top 3 best Primer pairs shown on a single page for convenience.
3. Primaclade and ABI Prisms' Primer Express was initially used for the primer design.
4. Individual Primer pair Blast results in single file is in the Folder "Blast Hits for Primer Rab11".

Only those primer pairs were selected where the forward and reverse primers picked up the same target gene for which the pair was designed with 100% hit coverage, score and identity. The primers found unique and not hitting other sequences in the same groups were screened and tested. The results of RT-PCR are shown in the gel image (fig.6.1). Section 6.3.2 shows a typical example of primers obtained by the Primer3 software with the primer sequences aligned to the target (TC199565/ LeRab11a). For the complete list of primers and the target sequence alignment please see the supplemental files as stated in point 4 above. Blast Report of Tomato Rab11/RabA Primers in TIGR DFCI TGI Database is shown below. Only top hits and best fit primer pairs are shown with their corresponding hits in TIGR DFCI TGI with the target TC ids of Rab GTPase.

1 .Query TC192047fwd1_(20 letters)
>tomato|TC192047 homologue to UniRef100_Q40522 Cluster: Ras-related protein Rab11D; n=1; Nicotiana tabacum|Rep: Ras-related protein Rab11D - Nicotiana tabacum (Common tobacco), complete Length = 967 Plus Strand HSPs: Score = 100 (21.1 bits), Expect = 52., P = 1.00000 Identities = 20/20 (100%), Positives = 20/20 (100%), Strand = Plus / Plus
Query: 1 GACTGAGGACAACAGCAGCA 20
Sbjct: 781 GACTGAGGACAACAGCAGCA 800

Query TC192047rvr1_(20 letters)
>tomato|TC192047 homologue to UniRef100_Q40522 Cluster: Ras-related protein Rab11D; n=1; Nicotiana tabacum|Rep: Ras-related protein Rab11D - Nicotiana tabacum (Common tobacco), complete Length = 967 Plus Strand HSPs: Score = 100 (21.1 bits), Expect = 52., P = 1.00000 Identities = 20/20 (100%), Positives = 20/20 (100%), Strand = Plus / Plus
Query 1 ATTTTCTCGGGTTGCAATTG 20 |
Sbjct: 855 ATTTTCTCGGGTTGCAATTG 874

2Query TC193549fwd4_(20 letters)
>tomato|TC193549 homologue to UniRef100_A7QPP6 Cluster: Chromosome chr10 scaffold_138, whole genome shotgun sequence; n=1; Vitis vinifera|Rep: Chromosome chr10 scaffold_138, whole genome shotgun sequence - Vitis vinifera (Grape), partial (98%) Length = 1014 Plus Strand HSPs: Score = 100 (21.1 bits), Expect = 53., P = 1.00000 Identities = 20/20 (100%), Positives = 20/20 (100%), Strand = Plus / Plus
Query: 1 GAGCTTCCCCTTGAGTTCCT 20
Sbjct: 862 GAGCTTCCCCTTGAGTTCCT 881
Query TC193549rvr4-(20 letters)
>tomato|TC193549 homologue to UniRef100_A7QPP6 Cluster: Chromosome chr10 scaffold_138, whole genome shotgun sequence; n=1; Vitis vinifera|Rep: Chromosome chr10 scaffold_138, whole genome shotgun sequence - Vitis vinifera (Grape), partial (98%) Length = 1014 Plus Strand HSPs: Score = 100 (21.1 bits), Expect = 53., P = 1.00000 Identities = 20/20 (100%), Positives = 20/20 (100%), Strand = Plus / Plus
Query: 1 TGGATGGGGTGAATTTTGAT 20
Sbjct: 958 TGGATGGGGTGAATTTTGAT 977

Query TC195610fwd1_(20 letters)
>tomato|TC195610 homologue to UniRef100_Q40521 Cluster: Ras-related protein Rab11B; n=1; Nicotiana tabacum|Rep: Ras-related protein Rab11B -Nicotiana tabacum (Common tobacco), complete Length = 1000 Plus Strand HSPs:
Score =100 (21.1 bits), Expect = 53., P = 1.00000 Identities = 20/20 (100%), Positives = 20/20 (100%), Strand = Plus / Plus
Query: 1 CAAGGCCTTTGCTGAAAGAG 20
Sbjct:518 CAAGGCCTTTGCTGAAAGAG 537
Query TC195610rvr1_(20 letters)
>tomato|TC195610 homologue to UniRef100_Q40521 Cluster: Ras-related protein Rab11B; n=1; Nicotiana tabacum|Rep: Ras-related protein Rab11B -Nicotiana tabacum (Common tobacco), complete Length = 1000 Plus Strand HSPs: Score = 100 (21.1 bits), Expect = 53., P = 1.00000 Identities = 20/20 (100%), Positives = 20/20 (100%), Strand = Plus / Plus
Query: 1 CACTCGAAGTTGGGGATGAT 20
Sbjct: 640 CACTCGAAGTTGGGGATGAT 659

Query TC195734 fwd1 (20 letters)
>tomato|TC195734 homologue to UniRef100_Q39434 Cluster: Ras-related protein Rab2BV; n=1; Beta vulgaris|Rep: Ras-related protein Rab2BV - Beta vulgaris (Sugar beet), complete
Query TC195734rvr4__(20 letters)
>tomato|TC195734 homologue to UniRef100_Q39434 Cluster: Ras-related protein Rab2BV; n=1; Beta vulgaris|Rep: Ras-related protein Rab2BV – Beta vulgaris (Sugar beet), complete Length = 747 Plus Strand HSPs: Score = 100 (21.1 bits), Expect = 49., P = 1.00000 Identities = 20/20 (100%), Positives = 20/20 (100%), Strand = Plus / Plus
Query: 1 TTCCTGGTCAGGGTACAACC 20
Sbjct: 631 TTCCTGGTCAGGGTACAACC 650

Query TC198194fwd1_ok (20 letters)
>tomato|TC198194 homologue to UniRef100_Q40524 Cluster: Nt-Rab11e protein; n=1; Nicotiana tabacum|Rep: Nt-Rab11e protein - Nicotiana tabacum (Common tobacco), complete Length = 903 Plus Strand HSPs: Score = 100 (21.1 bits), Expect = 52., P = 1.00000 Identities = 20/20 (100%), Positives = 20/20 (100%), Strand = Plus / Plus
Query: 1 TGCTTTTGGTGCTCTTGTTG 20
Sbjct: 327 TGCTTTTGGTGCTCTTGTTG 346
Query TC198194rvr1_ok (20 letters)
>tomato|TC198194 homologue to UniRef100_Q40524 Cluster: Nt-Rab11e protein; n=1; Nicotiana tabacum|Rep: Nt-Rab11e protein - Nicotiana tabacum (Common tobacco), complete Length = 903 Plus Strand HSPs: Score = 100 (21.1 bits), Expect = 52., P = 1.00000 Identities = 20/20 (100%), Positives = 20/20 (100%), Strand = Plus / Plus
Query: 1 GCAAGGATACTGGTGGGAAA 20
Sbjct: 430 GCAAGGATACTGGTGGGAAA 449

Query TC201753fwd1_(20 letters)
>tomato|TC201753 similar to UniRef100_Q40197 Cluster: RAB11G; n=1; Lotus japonicus|Rep: RAB11G - Lotus japonicus, partial (81%) Length = 705 Plus Strand HSPs: Score = 100 (21.1 bits), Expect = 48., P = 1.00000 Identities = 20/20 (100%), Positives = 20/20 (100%), Strand = Plus / Plus
Query: 1 TACCACAGTTGCGAGAATGC 20
Sbjct: 486 TACCACAGTTGCGAGAATGC 505
Query TC201753rvr2_(20 letters)
>tomato|TC201753 similar to UniRef100_Q40197 Cluster: RAB11G; n=1; Lotus japonicus|Rep: RAB11G - Lotus japonicus, partial (81%) Length = 705 Plus Strand HSPs: Score = 100 (21.1 bits), Expect = 48., P = 1.00000 Identities = 20/20 (100%), Positives = 20/20 (100%), Strand = Plus / Plus
Query: 1 GTTTTGCCCGTGATGAATTT 20
Sbjct: 233 GTTTTGCCCGTGATGAATTT 252

Query TC202127fwd1_(20 letters)
>tomato|TC202127 homologue to UniRef100_Q08149 Cluster: GTP-binding protein; n=1; Pisum sativum|Rep: GTP-binding protein - Pisum sativum (Garden pea), complete Length = 905 Plus Strand HSPs: Score = 100 (21.1 bits), Expect = 52., P = 1.00000 Identities = 20/20 (100%), Positives = 20/20 (100%), Strand = Plus / Plus
Query: 1 GTTGGAGGCAAGGATGATGT 20
Sbjct: 715 GTTGGAGGCAAGGATGATGT 734
Query TC202127rvr1_(20 letters)
>tomato|TC202127 homologue to UniRef100_Q08149 Cluster: GTP-binding protein; n=1; Pisum sativum|Rep: GTP-binding protein - Pisum sativum (Garden pea), complete Length = 905 Plus Strand HSPs: Score = 100 (21.1 bits), Expect = 52., P = 1.00000 Identities = 20/20 (100%), Positives = 20/20 (100%), Strand = Plus / Plus
Query: 1 TGAAGTAGGAGATGACCCGG 20
Sbjct: 666 TGAAGTAGGAGATGACCCGG 685

Query= TC202193_ (20 letters)
>tomato|TC202193 homologue to UniRef100_Q39434 Cluster: Ras-related protein Rab2BV; n=1; Beta vulgaris|Rep: Ras-related protein Rab2BV – Beta vulgaris (Sugar beet), partial (95%) length = 743 Plus Strand HSPs: Score = 100 (21.1 bits), Expect = 49., P = 1.00000 Identities = 20/20 (100%), Positives = 20/20 (100%), Strand = Plus / Plus
Query: 1 GTGGCTCCGAGAATTGAGAG 20
Sbjct: 429 GTGGCTCCGAGAATTGAGAG 448

Query= TC202193_rvr_(20 letters)
>tomato|TC202193 homologue to UniRef100_Q39434 Cluster: Ras-related protein Rab2BV; n=1; Beta vulgaris|Rep: Ras-related protein Rab2BV – Beta vulgaris (Sugar beet), partial (95%) Length = 743 Plus Strand HSPs: Score = 100 (21.1 bits), Expect = 49., P = 1.00000 Identities = 20/20 (100%), Positives = 20/20 (100%), Strand = Plus / Plus
Query: 1 CCTTAACCACCTTCGAGCAG 20
Sbjct: 498 CCTTAACCACCTTCGAGCAG 517
Query TC204406fwd4 OK_(20 letters)
>tomato|TC204406 similar to UniRef100_A7QC87 Cluster: Chromosome undetermined scaffold_77, whole genome shotgun sequence; n=1; Vitis vinifera|Rep: Chromosome undetermined scaffold_77, whole genome shotgun sequence - Vitis vinifera (Grape), complete Length = 919 Plus Strand HSPs: Score = 100 (21.1 bits), Expect = 52., P = 1.00000 Identities = 20/20 (100%), Positives = 20/20 (100%), Strand = Plus / Plus
Query: 1 TTGTTGTTGGTGCAGAGGAG 20
Sbjct: 713 TTGTTGTTGGTGCAGAGGAG 732
Query TC204406rvr4 OK (20 letters)
>tomato|TC204406 similar to UniRef100_A7QC87 Cluster: Chromosome undetermined scaffold_77, whole genome shotgun sequence; n=1; Vitis vinifera|Rep: Chromosome undetermined scaffold_77, whole genome shotgun sequence - Vitis vinifera (Grape), complete Length = 919 Plus Strand HSPs: Score = 100 (21.1 bits), Expect = 52., P = 1.00000 Identities = 20/20 (100%), Positives = 20/20 (100%), Strand = Plus / Plus
Query: 1 ATGATTCCTCGCTTGCTGTT 20
Sbjct: 768 ATGATTCCTCGCTTGCTGTT 787
Query TC205638fwd1_ (20 letters)
>tomato|TC205638 homologue to UniRef100_A7QC87 Cluster: Chromosome undetermined scaffold_77, whole genome shotgun sequence; n=1; Vitis vinifera|Rep: Chromosome undetermined scaffold_77, whole genome shotgun sequence - Vitis vinifera (Grape), partial (73%) Length = 678 Plus Strand HSPs: Score = 100 (21.1 bits), Expect = 47., P = 1.00000 Identities = 20/20 (100%), Positives = 20/20 (100%), Strand = Plus / Plus
Query: 1 ACGGCTGGACAGGAGAGATA 20
Sbjct: 407 ACGGCTGGACAGGAGAGATA 426
Query TC205638rvr1_(20 letters)
>tomato|TC205638 homologue to UniRef100_A7QC87 Cluster: Chromosome undetermined scaffold_77, whole genome shotgun sequence; n=1; Vitis vinifera|Rep: Chromosome undetermined scaffold_77, whole genome shotgun sequence - Vitis vinifera (Grape), partial (73%) Length = 678 Plus Strand HSPs: Score = 100 (21.1 bits), Expect = 47., P = 1.00000 Identities = 20/20 (100%), Positives = 20/20 (100%), Strand = Plus / Plus
Query: 1 ATGTGAGCCGGTGGTTAAAG 20
Sbjct: 510 ATGTGAGCCGGTGGTTAAAG 529
Query TC207026fwd4_(20 letters)
>tomato|TC207026 homologue to UniRef100_Q39434 Cluster: Ras-related protein Rab2BV; n=1; Beta vulgaris|Rep: Ras-related protein Rab2BV - Beta vulgaris (Sugar beet), partial (72%) Length = 522
Plus Strand HSPs: Score = 100 (21.1 bits), Expect = 43., P = 1.00000 Identities = 20/20 (100%), Positives = 20/20 (100%), Strand = Plus / Plus
Query: 1 ATGTTCTGAGGTGGCTACGG 20
Sbjct: 361 ATGTTCTGAGGTGGCTACGG 380
Query TC207026rvr4_(20 letters)
>tomato|TC207026 homologue to UniRef100_Q39434 Cluster: Ras-related protein Rab2BV; n=1; Beta vulgaris|Rep: Ras-related protein Rab2BV – Beta vulgaris (Sugar beet), partial (72%) Length = 522 Plus Strand HSPs: Score = 100 (21.1 bits), Expect = 43., P = 1.00000 Identities = 20/20 (100%), Positives = 20/20 (100%), Strand = Plus / Plus
Query: 1 TGAGAAGGAAGGGCTGTCAT 20
Sbjct: 488 TGAGAAGGAAGGGCTGTCAT 507
Query TC207267fwd1_(20 letters)
>tomato|TC207267 homologue to UniRef100_A7QDG9 Cluster: Chromosome chr10 scaffold_81, whole genome shotgun sequence; n=1; Vitis vinifera|Rep: Chromosome chr10 scaffold_81, whole genome shotgun sequence - Vitis vinifera (Grape), partial (96%) Length = 1060 Plus Strand HSPs: Score = 100 (21.1 bits), Expect = 53., P = 1.00000 Identities = 20/20 (100%), Positives = 20/20 (100%), Strand = Plus / Plus
Query: 1 AGAATGGAAACGGAAACGTG 20
Sbjct: 730 AGAATGGAAACGGAAACGTG 749
Query TC207267rvr1_(20 letters)
>tomato|TC207267 homologue to UniRef100_A7QDG9 Cluster: Chromosome chr10 scaffold_81, whole genome shotgun sequence; n=1; Vitis vinifera|Rep: Chromosome chr10 scaffold_81, whole genome shotgun sequence - Vitis vinifera (Grape), partial (96%)Length = 1060 Plus Strand HSPs:Score = 100 (21.1 bits), Expect = 53., P = 1.00000 Identities = 20/20 (100%), Positives = 20/20 (100%), Strand = Plus / Plus
Query: 1 ATCCTGTTCTAGGTGCACCG 20
Sbjct: 851 ATCCTGTTCTAGGTGCACCG 870
Query TC207267fwd1_(20 letters)
>tomato|TC207551 homologue to UniRef100_Q9SPK2 Cluster: Rab GTP-binding protein Zab11a; n=1; Gossypium hirsutum|Rep: Rab GTP-binding protein Rab11a - Gossypium hirsutum (Upland cotton) (Gossypium mexicanum), partial (84%) Length = 1197 Plus Strand HSPs:Score = 100 (21.1 bits), Expect = 55., P = 1.00000 Identities = 20/20 (100%), Positives = 20/20 (100%), Strand = Plus / Plus
Query: 1 TTTGATCACATCGTGGGAGA 20
Sbjct: 137 TTTGATCACATCGTGGGAGA 156
Query TC207551rvr1 _(20 letters)
>tomato|TC207551 homologue to UniRef100_Q9SPK2 Cluster: Rab GTP-binding protein Rab11a; n=1; Gossypium hirsutum|Rep: Rab GTP-binding protein Rab11a - Gossypium hirsutum (Upland cotton) (Gossypium mexicanum), partial
(84%) Length = 1197 Plus Strand HSPs:Score = 100 (21.1 bits), Expect = 55., P = 1.00000 Identities = 20/20 (100%), Positives = 20/20 (100%), Strand = Plus / Plus
Query: 1 GGTCTGCAAAAGAAGCAAGG 20
Sbjct: 212 GGTCTGCAAAAGAAGCAAGG 231
Query= TC207615fwd1_(20 letters)
>tomato|TC207615 homologue to UniRef100_A7QPP6 Cluster: Chromosome chr10 scaffold_138, whole genome shotgun sequence; n=1; Vitis vinifera|Rep: Chromosome chr10 scaffold_138, whole genome shotgun sequence - Vitis vinifera (Grape), partial (82%) Length = 759 Plus Strand HSPs:Score = 100 (21.1 bits), Expect = 49., P = 1.00000 Identities = 20/20 (100%), Positives = 20/20 (100%), Strand = Plus / Plus
Query: 1 CAAGGTGGTGTTGATTGGTG 20
Sbjct: 250 CAAGGTGGTGTTGATTGGTG 269
Query TC207615rvr1_(20 letters)
>tomato|TC207615 homologue to UniRef100_A7QPP6 Cluster: Chromosome chr10 scaffold_138, whole genome shotgun sequence; n=1; Vitis vinifera|Rep: Chromosome chr10 scaffold_138, whole genome shotgun sequence - Vitis vinifera (Grape), partial (82%) Length = 759

Plus Strand HSPs:Score = 100 (21.1 bits), Expect = 49., P = 1.00000 Identities = 20/20 (100%), Positives = 20/20 (100%), Strand = Plus / Plus
Query: 1 TGAGTTCGCTACTCGAAGCA 20
Sbjct: 352 TGAGTTCGCTACTCGAAGCA 371
Query TC208438fwd1 ok (20 letters)
>tomato|TC208438 homologue to UniRef100_Q40522 Cluster: Ras-related protein Rab11D; n=1; Nicotiana tabacum|Rep: Ras-related protein Rab11D - Nicotiana tabacum (Common tobacco), complete Length = 1012 Plus Strand HSPs:Score = 100 (21.1 bits), Expect = 53., P = 1.00000 Identities = 20/20 (100%), Positives = 20/20 (100%), Strand = Plus / Plus
Query: 1 CCACCGAGGATGCTAAAGAA 20
Sbjct: 598 CCACCGAGGATGCTAAAGAA 617
Query TC208438rvr1 ok (20 letters)
>tomato|TC208438 homologue to UniRef100_Q40522 Cluster: Ras-related protein Rab11D; n=1; Nicotiana tabacum|Rep: Ras-related protein Rab11D -Nicotiana tabacum (Common tobacco), complete Length = 1012 Plus Strand HSPs:Score = 100 (21.1 bits), Expect = 53., P = 1.00000 Identities = 20/20 (100%), Positives = 20/20 (100%), Strand = Plus / Plus
Query: 1 GAACAAGAAGAATCTCGCCG 20
Sbjct: 722 GAACAAGAAGAATCTCGCCG 741
Query TC208692fwd1 ok (20 letters)
>tomato|TC208692 similar to UniRef100_Q40200 Cluster: RAB11J; n=1; Lotus japonicus|Rep: RAB11J - Lotus japonicus, partial (96%) Length = 1464 Plus Strand HSPs:Score = 100 (21.1 bits), Expect = 56., P = 1.00000 Identities = 20/20 (100%), Positives = 20/20 (100%), Strand = Plus / Plus
Query: 1 TTCCACCACTCCTCATCTCC 20
Sbjct: 458 TTCCACCACTCCTCATCTCC 477
Query TC208692rvr1 ok (20 letters)
>tomato|TC208692 similar to UniRef100_Q40200 Cluster: RAB11J; n=1; Lotus japonicus|Rep: RAB11J - Lotus japonicus, partial (96%) Length = 1464 Plus Strand HSPs:Score = 100 (21.1 bits), Expect = 56., P = 1.00000 Identities = 20/20 (100%), Positives = 20/20 (100%), Strand = Plus / Plus
Query: 1 GCTGTTTCCTTCTGTAGCCG 20
Sbjct: 521 GCTGTTTCCTTCTGTAGCCG 540

Query TC211111fwd1 OK(20 letters)
>tomato|TC211111 homologue to UniRef100_A7QPP6 Cluster: Chromosome chr10 scaffold_138, whole genome shotgun sequence; n=1; Vitis vinifera|Rep: Chromosome chr10 scaffold_138, whole genome shotgun sequence - Vitis vinifera (Grape), partial (71%) Length = 788 Plus Strand HSPs:Score = 100 (21.1 bits), Expect = 50., P = 1.00000 Identities = 20/20 (100%), Positives = 20/20 (100%), Strand = Plus / Plus
Query: 1 GCGTTGTTAGCAGGAAAAGC 20
Sbjct: 342 GCGTTGTTAGCAGGAAAAGC 361
Query TC211111rvr1_(20 letters)
>tomato|TC211111 homologue to UniRef100_A7QPP6 Cluster: Chromosome chr10 scaffold_138, whole genome shotgun sequence; n=1; Vitis vinifera|Rep: Chromosome chr10 scaffold_138, whole genome shotgun sequence - Vitis vinifera (Grape), partial (71%) Length = 788 Plus Strand HSPs:Score = 100 (21.1 bits), Expect = 50., P = 1.00000 Identities = 20/20 (100%), Positives = 20/20 (100%), Strand = Plus / Plus
Query: 1 AAATGTTGGAGGCAAGGATG 20
Sbjct: 410 AAATGTTGGAGGCAAGGATG 429
Query TC211213fwd1 OK (20 letters)
>tomato|TC211213 similar to UniRef100_Q08149 Cluster: GTP-binding protein; n=1; Pisum sativum|Rep: GTP-binding protein - Pisum sativum (Garden pea), complete Length = 872 Plus Strand HSPs: Score = 100 (21.1 bits), Expect = 51., P = 1.00000 Identities = 20/20 (100%), Positives = 20/20 (100%), Strand = Plus / Plus
Query: 1 GCAGTGGGTGCCTTACTTGT 20
Sbjct: 400 GCAGTGGGTGCCTTACTTGT 419
Query TC211213rvr1 OK (20 letters)
>tomato|TC211213 similar to UniRef100_Q08149 Cluster: GTP-binding protein; n=1; Pisum sativum|Rep: GTP-binding protein - Pisum sativum (Garden pea), complete Length = 872 Plus Strand HSPs:Score = 100 (21.1 bits), Expect = 51., P = 1.00000 Identities = 20/20 (100%), Positives = 20/20 (100%), Strand = Plus / Plus
Query: 1 CCACACAGACCAAAACATCG 20
Sbjct: 483 CCACACAGACCAAAACATCG 502
Query TC212051fwd2n5_ (20 letters)
>tomato|TC212051 similar to UniRef100_Q39860 Cluster: GTPase; n=1; Glycine max|Rep: GTPase - Glycine max (Soybean), partial (87%) Length = 873 Pus Strand HSPs:Score = 100 (21.1 bits), Expect = 51., P = 1.00000 Identities = 20/20 (100%), Positives = 20/20 (100%), Strand = Plus / Plus
Query: 1 GAAACATCGGCTCTCGACTC 20
Sbjct: 448 GAAACATCGGCTCTCGACTC 467
Query TC212051rvr2 ok (20 letters)
>tomato|TC212051 similar to UniRef100_Q39860 Cluster: GTPase; n=1; Glycine max|Rep: GTPase - Glycine max (Soybean), partial (87%) Length = 873 Plus Strand HSPs:Score = 100 (21.1 bits), Expect = 51., P = 1.00000 Identities = 20/20 (100%), Positives = 20/20 (100%), Strand = Plus / Plus
Query: 1 ATCTGTCAATCGCGTTAGCC 20
Sbjct: 564 ATCTGTCAATCGCGTTAGCC 583

Query= TC199565_fwd1_(20 letters)
>tomato|TC199565 UniRef100_Q9SCB1 Cluster: Rab11 GTPase; n=1; Solanumlycopersicum|Rep: Rab11 GTPase - Solanum lycopersicum (Tomato) (Lycopersicon esculentum), complete Length = 1206 Plus Strand HSPs: Score = 100 (21.1 bits), Expect = 55., P = 1.00000 Identities = 20/20 (100%), Positives = 20/20 (100%), Strand = Plus / Plus
Query: 1 AAAAGTTTGGGTGCTGTTCG 20
Sbjct: 739 AAAAGTTTGGGTGCTGTTCG 758
Query= TC199565rvr1__(20 letters)
>tomato|TC199565 UniRef100_Q9SCB1 Cluster: Rab11 GTPase; n=1; Solanum lycopersicum|Rep: Rab11 GTPase - Solanum lycopersicum (Tomato) (Lycopersicon esculentum), complete Length = 1206 Plus Strand HSPs:Score = 100 (21.1 bits), Expect = 55., P = 1.00000 Identities = 20/20 (100%), Positives = 20/20 (100%), Strand = Plus / Plus
Query: 1 CTCACTTTGGGTATCGCGAT 20
Sbjct: 847 CTCACTTTGGGTATCGCGAT 866

6.3.2 Example of Primer picking results for TC199565/*LeRab11a*

```
No mispriming library specified       Using 1-based sequence positions
OLIGO            start  len      tm     gc%   any   3' seq
LEFT PRIMER        739   20   60.15   45.00  5.00  2.00 AAAAGTTTGGGTGCTGTTCG
RIGHT PRIMER       866   20   60.10   50.00  8.00  1.00 ATCGCGATACCCAAAGTGAG
SEQUENCE SIZE: 1206          INCLUDED REGION SIZE: 1206
PRODUCT SIZE: 128, PAIR ANY COMPL: 7.00, PAIR 3' COMPL: 0.00
    1 AAGAATTTATTTTTTCTTCTTCAAAATTGAATCTTTTCAGTGAACTTTTACTATTTGGT

   61 CAATCAAGAAGTGAAATTACAGAGCAAAAGCAGATATAGTTTTGAAGATGGCAGGTTACA

  121 GAGGTGATGATGAGTATGATTACCTTTTCAAGCTTGTGTTGATAGGTGATTCAGGTGTGG

  181 GTAAATCTAATCTGCTTTCCAGGTTCACCAAAAATGAATTCAATTTGGAGTCTAAATCAA

  241 CTATTGGGGTTGAATTTGCAACCAAAAGTCTTAATATTGATGGTAAAGTTATTAAAGCTC

  301 AGATTTGGGATACTGCTGGTCAAGAAAGGTATCGTGCCATTACGAGTGCTTATTATCGGG

  361 GAGCTGTCGGTGCTTTGCTCGTATATGATGTTACTCGACATGTTACCTTTGAAAACGTCA

  421 ACAGGTGGTTGAAGGAATTGAGAGACCATACTGACCCTAACATTGTAGTCATGCTCCTAG

  481 GCAACAAATCAGATCTCCGACATCTCGTGGCTGTTTCAACTGAAGAGGCTAAATCTTTGG

  541 CAGAGAGGGAAGCCCTATACTTCATGGAAACTTCTGCATTAGAAGCGACCAACGTTGAAA

  601 ATGCGTTTACTGAAGCTCTCACGCAGATATACCGGATCGTCAGTAAGAAAGCAGTCGAGG

  661 CAGGGGACGAAGGTGCTACTTCAACCGCACCACCTAAAGGACAGACGATTAATATCAAAG

  721 ATGAAGGATCTGCTTGGAAAAAGTTTGGGTGCTGTTCGAGCTAGGGCTCACGTAAGACAA
                     >>>>>>>>>>>>>>>>>>>
  781 TAGCTCAAGCTTTATATATCAGTTGTAAATTGTCATTAAATCAATGATTTCCTCTCCCTG

  841 TTTTCTCTCACTTTGGGTATCGCGATTGTGGGATATAAGATTTATTCTATAGTCATGAGA
           <<<<<<<<<<<<<<<<<<<
  901 ATACGTGAAGCAGCGCGTTCTCATGAAAAATTCCGAAGGAGGATCAGGTACTGCTAGTTT

  961 CTAATTACAGAATGTCTAAACAAGGTCTTAGTTTTATGTTTTCTGGTTGATGGCTTGAAA

 1021 AACTGGAGATATCTTTGCAATAACCTGTTACCGAATATCTTCTTATTTGTCTCGAATTTA

 1081 CGTCTTAATTATTTTTCCCCTTCTAAAAGTGTATTTCCTGAGCTGAGTAGCAATGTTTGT

 1141 TGTTCATGATATGTGTAGTTTGATAAATTAAAGTAGAACATGGCAAGGTGTGCCCAGAGT

 1201 TGATTT
KEYS (in order of precedence):>>>>>> left primer<<<<<< right primer
ADDITIONAL OLIGOS
                   start  len      tm     gc%   any   3' seq
 1 LEFT PRIMER       741   20   60.29   50.00  4.00  2.00 AAGTTTGGGTGCTGTTCGAG
   RIGHT PRIMER      866   20   60.10   50.00  8.00  1.00 ATCGCGATACCCAAAGTGAG
   PRODUCT SIZE: 126, PAIR ANY COMPL: 7.00, PAIR 3' COMPL: 2.00
 2 LEFT PRIMER       499   20   59.84   50.00  4.00  1.00 GACATCTCGTGGCTGTTTCA
   RIGHT PRIMER      595   20   60.28   50.00  4.00  2.00 ACGTTGGTCGCTTCTAATGC
   PRODUCT SIZE: 97, PAIR ANY COMPL: 3.00, PAIR 3' COMPL: 1.00
 3 LEFT PRIMER       676   20   60.17   55.00  3.00  0.00 CTACTTCAACCGCACCACCT
   RIGHT PRIMER      760   20   60.29   50.00  4.00  3.00 CTCGAACAGCACCCAAACTT
   PRODUCT SIZE: 85, PAIR ANY COMPL: 3.00, PAIR 3' COMPL: 0.00
 4 LEFT PRIMER       350   20   60.05   50.00  4.00  2.00 TTATTATCGGGGAGCTGTCG
   RIGHT PRIMER      428   20   59.58   45.00  4.00  3.00 CCACCTGTTGACGTTTTCAA
   PRODUCT SIZE: 79, PAIR ANY COMPL: 3.00, PAIR 3' COMPL: 1.00
Statistics
        con   too    in    in          no    tm    tm  high  high        high
        sid  many   tar  excl   bad    GC   too   too   any    3'  poly   end
       ered    Ns   get   reg   GC% clamp   low  high compl compl     X  stab
ok
Left   10533     0     0     0   248     0  5148  2144     1    16     3   115
2858
Right  10535     0     0     0   194     0  5107  2250     0     3     0   112
2869Pair Stats: considered 810, unacceptable product size 789, high
end compl 3, ok 18
```

6.3.3 Digital PCR/*in silico* PCR of Tomato Rab11 Genes with the Primers in SGN Database

The table 6.3 shows the digital PCR/*in silico* PCR of Tomato Rab11 GTPase genes with the primers designed by Primer3 software in SGN database. This is yet another approach to validate the primers that were designed for the present study. The primer pairs were individually used in the BLAST search in SGN database to get the corresponding hits of SGN-Unigene-Id numbers which were then correlated to the previously designed and compiled database of TIGR TCs and SGN Unigene ids (Chapter 3, Table no.3.12).

Most of the Primers pairs fit the unique criteria of hitting the target sequence only. The table also shows the target hit details, their strand-start and strand-end size. The results were also correlated to the latest release of the SolycIds from the tomato whole genome sequence release ITAG2.4 in SGN database.

The table 6.3 shows the TIGR TC of Rab11 GTPase Release12 in the first column, followed by the primer pair (forward and reverse primer name), primer sequence and the corresponding Solyc Id hits with the annotation of Rab11 GTPases as well as the SGN-Unigene Id hits. In most cases as can be observed from the table the hits matched the unique sequences. The cases where the sequences could not be obtained a different approach of reverse blast to get the SGN unigene ids in order to get the main SolycIDs was applied. As a result of this approach it was possible to find and assign the unique SolycIDs where there was a gap between the TIGR-TC and SGN-Unigene IDs as was discussed in chapter 3 about the discrepancy of the different database being updated at different time since it was difficult to have a common consensus. But the development of the three earlier databases at every single update and release from TIGR-TC to SGN-Unigene IDs to TED database Probe ids to the final Solyc Ids bears fruits when the correlation fits well in the sequence homology and correspondence. The problem in working with the current Solyc Ids is many of the Solyc Ids do not show any unique SGN-Unigene associated with them. Thus the reverse approach was used to Blast the TIGR-TC to find SGN ids and then getting the main Solyc Ids to be sure of the primers picking unique sequences.

Table 6.3 Digital PCR/*in silico* PCR of Tomato Rab11 Genes with the Primers in SGN Database

Genes	Name of Primers	Primer Sequences Used for Blast	Digital PCR Solo Hits	Rab11	SGN Unigene	Product Hit Details Strand-Start-End-Size
TC195734	TC195734fwd TC195734rvr	TTCTTGAGACATCGGCATTG GGTTGTACCCTGACCAGGAA	Solyc06g005810.2.1	AtRABA2b	SGN-U569324	> Solyc06g005810.2.1 strand = plus, start = 579, end = 724, size = 146 bp
TC193549	TC193549fwd TC193549rvr	GAGCTTCCCCTTGAGTTCCT ATCAAAATTCACCCCATCCA	Solyc07g053480	AtRABA1f	SGN-U577848	> Solyc07g053480.2.1 strand = plus, start = 892, end = 1007, size = 116 bp
TC212461	TC212461fwd TC212461rvr	TGTTGGAGATTCAGCTGTGG TCCCATATTTGAGCCTTGATG	Solyc03g118820.2	AtRABA6a	SGN-U577717	> Solyc03g118820.2.1 strand = plus, start = 108, end = 257, size = 150 bp
TC192047	TC192047fwd TC192047rvr	GACTGAGGACAACAGCAGCA CAATTGCAACCCGAGAAAAT	Solyc04g012180	AtRABA4a	SGN-U569627	> Solyc04g012180.2.1 strand = plus, start = 896, end = 989, size = 94 bp
TC199565	TC199565fwd TC199565rvr	AAAAGTTTGGGTGCTGTTCG ATCGCGATACCCAAAGTGAG	Solyc07g055290.2	LeRab11a AtRABA1a	SGN-U564990	> Solyc07g055290.2.1 strand = plus, start = 766, end = 893, size = 128 bp
TC195610	TC195610fwd TC195610rvr	CAAGGCCTTTGCTGAAAGAG ATCATCCCCAACTTCGAGTG	Solyc12g014080.1	AtRABA1f	SGN-U564989	> Solyc12g014080.1.1 strand = plus, start = 426, end = 567, size = 142 bp
TC198194	TC198194fwd TC198194rvr	TGCTTTTGGTGCTCTTGTTG TTTCCCACCAGTATCCTTGC	Solyc09g098170	AtRABA5e	SGN-U564991	> Solyc09g098170.2.1 strand = plus, start = 372, end = 494, size = 123 bp
TC207026	TC207026fwd TC207026rvr	ATGTTCTGAGGTGGCTACGG ATGACAGCCCTTCCTTCTCA	Solyc06g076450.2	AtRABA2b	SGN-U569326	> Solyc06g076450.2.1 strand = plus, start = 455, end = 601, size = 147 bp
TC207267	TC207267fwd TC207267rvr	AGAATGGAAACGGAAACGTG CGGTGCACCTAGAACAGGAT	Solyc02g072180.2	AtRABA1d	SGN-U578944	> Solyc02g072180.2.1 strand = plus, start = 749, end = 889, size = 141 bp
TC204406	TC204406fwd TC204406rvr	TTGTTGTTGGTGCAGAGGAG AACAGCAAGCGAGGAATCAT	Solyc10g045550.1	AtRABA2a	SGN-U569325	Reverse Blast hits from TIGR TC to SGN Unigene & from SGN Unigene to Solyc IDs Confirmed

TC207551	TC207551fwd TC207551rvr	TTTGATCACATCGTGGGAGA CCTTGCTTCTTTTGCAGACC	Solyc11g012460.1	AtRABA5a	SGN-U564984	Reverse Blast hits from TIGR TC to SGN Unigene & from SGN Unigene to Solyc IDs Confirmed
TC208438	TC208438fwd TC208438rvr	CCACCGAGGATGCTAAAGAA CGGCGAGATTCTTCTTGTTC	Solyc11g010100.1	AtRABA4a	SGN-U569628	> Solyc11g010100.1.1 strand = plus, start = 422, end = 565, size = 144 bp
TC208692	TC208692fwd TC208692rvr	TTCCACCACTCCTCATCTCC CGGCTACAGAAGGAAACAGC	Solyc05g054150.2	AtRABA5a	SGN-U564985	> Solyc05g054150.2.1 strand = plus, start = 48, end = 130, size = 83 bp
TC212051	TC212051fwd TC212051rvr	GAAACATCGGCTCTCGACTC GGCTAACGCGATTGACAGAT	Solyc09g056340.2	AtRABA5b	SGN-U568799	Reverse Blast hits from TIGR TC to SGN Unigene & from SGN Unigene to Solyc IDs Confirmed
TC201753	TC201753fwd TC201753rvr	TAACCTTCCGCCCCTTTATT TCCCCAATCACCACAATCTT	Solyc09g056340.2	AtRABA5b	SGN-U568799	Reverse Blast hits from TIGR TC to SGN Unigene & from SGN Unigene to Solyc IDs Confirmed
TC211111	TC211111fwd TC211111rvr	GCGTTGTTAGCAGGAAAAGC CATCCTTGCCTCCAACATTT	Solyc12g010790.1	AtRABA1f	SGN-U577600	Reverse Blast hits from TIGR TC to SGN Unigene & from SGN Unigene to Solyc IDs Confirmed
TC202127	TC202127fwd TC202127rvr	GTTGGAGGCAAGGATGATGT AGAATCTCTTCCGCGGTGTA	Solyc12g010790.1	AtRABA1f	SGN-U577600	Reverse Blast hits from TIGR TC to SGN Unigene & from SGN Unigene to Solyc IDs Confirmed
TC211213	TC211213fwd TC211213rvr	GCAGTGGGTGCCTTACTTGT CGATGTTTTGGTCTGTGTGG	Solyc03g006270.2	AtRABA1f	SGN-U584956	> Solyc03g006270.2.1 strand = plus, start = 357, end = 459, size = 103 bp

The table above shows the corresponding TIGR TCs (Release12), SGN Unigene Ids (Build-2) and Solyc Ids (SGN ITAG2.4) for Tomato Rab11 GTPase group. The TIGR-TCs were correlated with SGN-unigene Ids and where the Solyc IDs were not found the Reverse BLAST was used to hunt the corresponding SolycIDs using the SGN Unigene Ids. The blue highlighted SGN-Unigene ids represent the cases where the corresponding Unigene Ids are listed along with the SolycIds in the SGN database. The rest of unigene ids were found by reverse blast to associate them with the new SolycIds. The fifth column represents the probable annotation of tomato Rab11 GTPase class of genes.

Table 6.4 Layout of the 384 well plate for the qRT-PCR experiment

			ACTIN	GAPDH	PGK	UBI	EFT	TC 195734	TC 193549	TC 212461	TC 192047	TC 199565	TC 195610	TC 198194	TC 207026	TC 207267	TC 204406	TC 207551	TC 208438	TC 208692	TC 212051	TC 201753	TC 211111	TC 202127	TC 211213	
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Gene	A	1	○																							
	B	2	○																							
	C	3	○																							
H_2O	D	4																								
	E	5																								
	F	6																								
RNA	G	7																								
	H	8																								
	I	9																								
	J	10																								
Gene	K	11																								
	L	12																								
	M	13																								
H_2O	N	14																								
	O	15																								
	P	16																								

6.4 Results of qRT-PCR

The data from the qRT-PCR experiment was analysed by using the relative delta Ct method according the formula by Perikles (2003) Livak and Schmittgen, (2001). Fig. 6.2 shows the entire expression profile of all *LeRab11* GTPase in wild type (AC++) fruits from the different developmental stages ranging from 7DPA, 15 DPA, 30DPA, Mature green, MG, Breaker (B) and B+10. These results indicate that at least three genes have distinct expression profiles and can be the putative candidate genes for the future work for Rab11 GTPases. The target Rab11a used for the generation of antisense transgenic lines 'TC199565' has an increased expression pattern from 7 DPA until the stage of mature green and breaker stages but after breaker and Breaker +10 the expression of TC199565 starts to decline as reported by Lu *et al*., (2001).

The three putative candidate genes that are expressed highly are TC199565 until mature green stage and the others are TC207026, TC208692 and TC202127. These can be used for further studies to elucidate their role in fruit ripening and fruit softening.

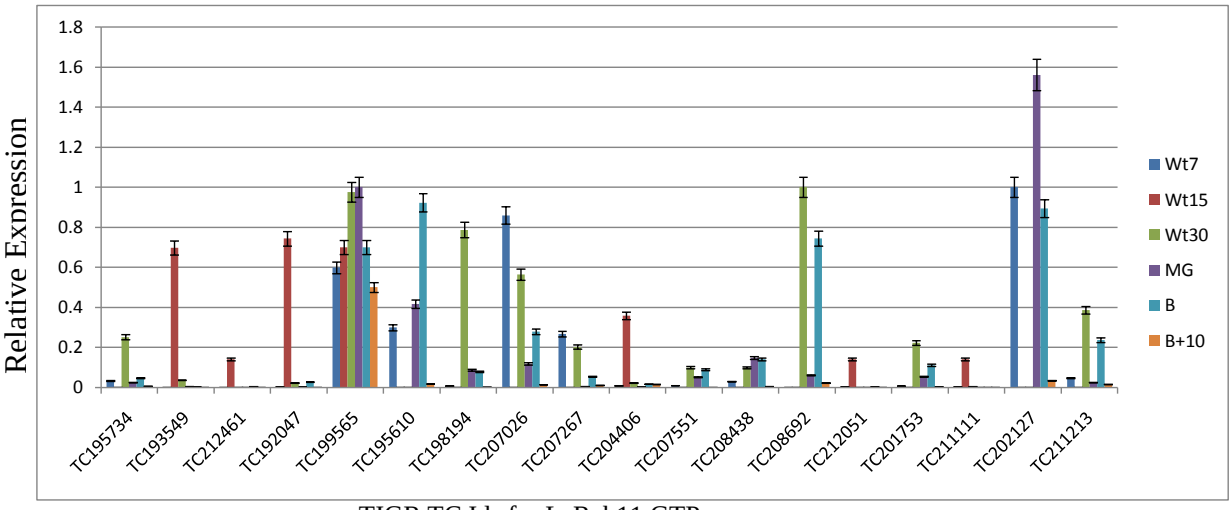
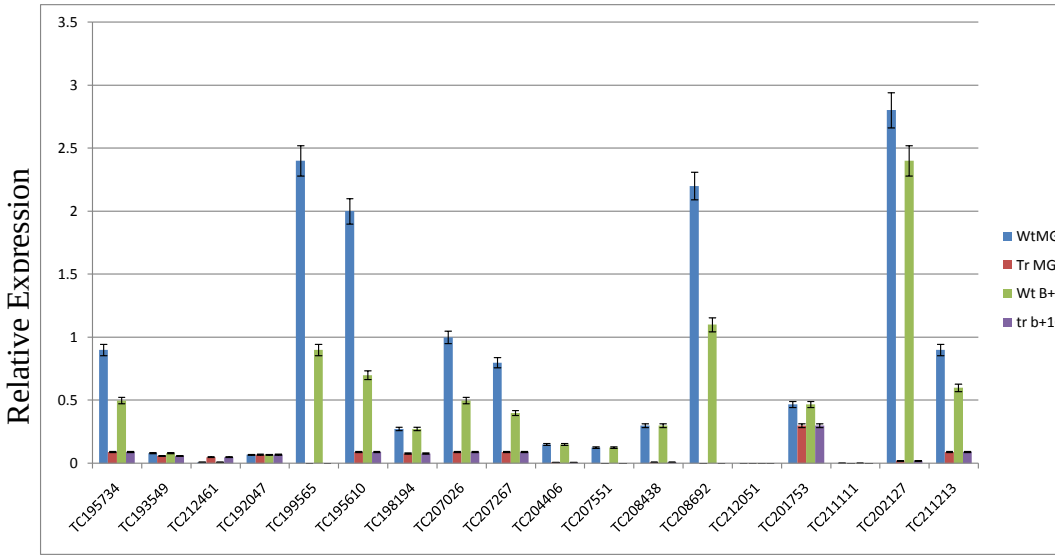


Fig.6.2: The Relative expression profile of LeRab11 GTPase in wild type (AC++) normalized with GAPDH as house keeping gene at different developmental stages of fruit ripening. Fig shows the relative gene expression pattern of 18 *LeRab11* GTPases in AC++ fruits (WT) at different developmental stages including 7 days after pollination (represented in the graph byWt7), 15DPA (Wt15), Wt30 immature green stage, Mature green stage (MG), Breaker (B) and Breaker +10 days (B+10). Error bars represent ±SE with n=3 replicates for each gene at each stage.



TIGR TC Ids for LeRab11 GTPase genes

Fig.6.3 Relative expression profile of LeRab11 GTPase genes at mature green and breaker +10 (B+10) stage in wild type (AC++) and transgenic (*LeRab11a* Antisense fruits)

As shown in fig.6.3 the same three genes TC199565, TC208692 and TC202127 are up regulated during the mature green (MG) stage whereas in case of the *Le Rab11a* antisense they are remarkably down regulated. This might indicate their function in trafficking of cell wall hydrolases or enzymes during the later stage of ripening. As these genes are down regulated in case of transgenic lines this might indicate the reason for the pattern observed in the delayed softening of the fruits from the transgenic plants (as observed and discussed in chapter 4, 5 and 7). To avoid complexity only the graphs showing the significant differences between the expression pattern of genes in wild type and transgenic fruits are shown.

It would be interesting to see if the same expression pattern can be observed in other ripening and non ripening mutants of tomato. The reason for more scattered profiles could be the result of compensation by the other group members of the same class of GTPases in the event of down regulation or complete inhibition of one particular Rab11GTPases. It might be possible in the case of LeRab11a antisense line which needs further investigation.

The expression profiles were also generated by analyzing the raw data from the TED database for wild type tomato (AC++) by downloading the raw files for which the expression data was available and could be assigned to the latest

SolycIds. The graph shows the expression profiles of different available Rab GTPase from Class1 to class18. This analysis helped in gaining a fair idea about the expression pattern at different stages of fruit development. The SolycIds represented here indicates the different Rab GTPases. In any Solyc id code *e.g.* Solyc01g103370.2.1, the initial solyc means the species *i.e. Solanum lycopersicum* followed by two digits representing the chromosome. In this case its 01 that means it is located on chromosome one which is then followed by the unique code and 2.1 which represents the version.

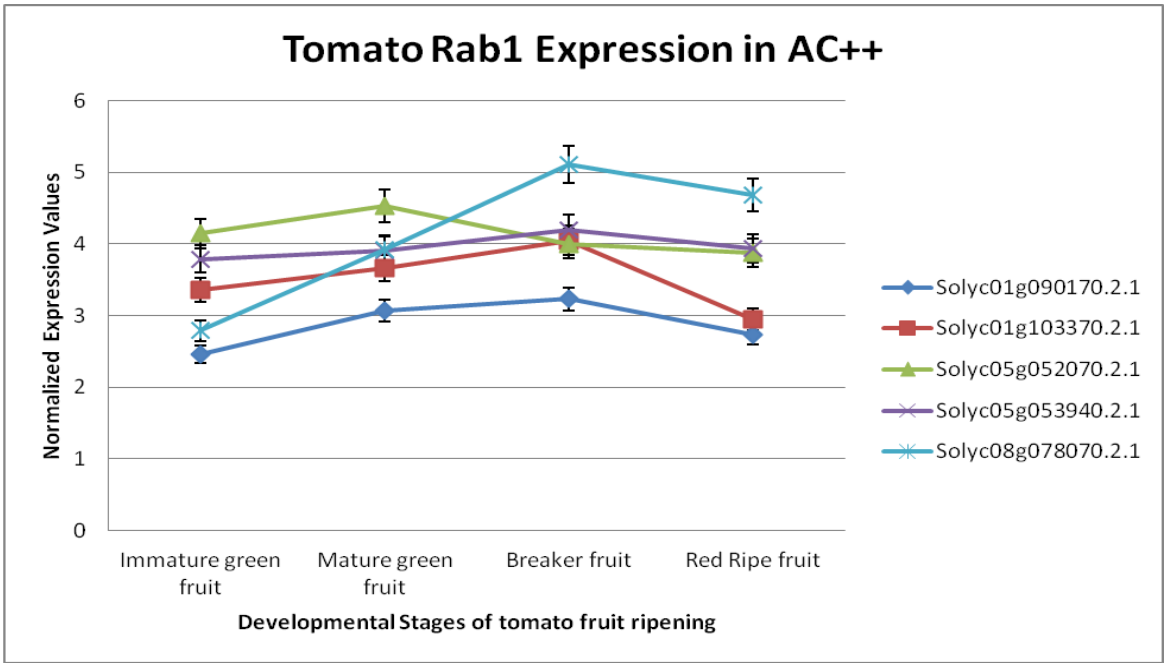


Fig.6.4: Relative expression profile of Rab1/RabD GTPase. The figure shows the gene expression pattern of five RabD/Rab1 GTPases at the Immature green (IMG), Mature Green (MG), Breaker (Brk) and fully ripe or Red ripe (RR) fruit of AC++.

The fig.6.4 shows the expression pattern of the Rab1/RabD GTPase in the wild type tomato AC++ at the different developmental stages i.e. at immature green (IMG), mature green (MG), breaker (Brk) and fully ripe or red ripe (RR) fruits. The expression of Solyc05g052070 increases as the fruit reaches maturity from immature green to mature green then declines at breaker and there is no significant rise in its expression in red ripe fruit. The three RabD/1 GTPase Solyco1g103370, Solyc05g052070 and Solyc05g053940 follow almost a similar pattern where there is a marginal increase in the expression from IMG, MG and Brk stages and then a partial decrease at red ripe stage except Solyc08g078070 which shows significant increase in the expression where the expression goes on

increasing at IMG to MG and from MG to Breaker stage and then a significant decrease at red ripe stage of fruit development.

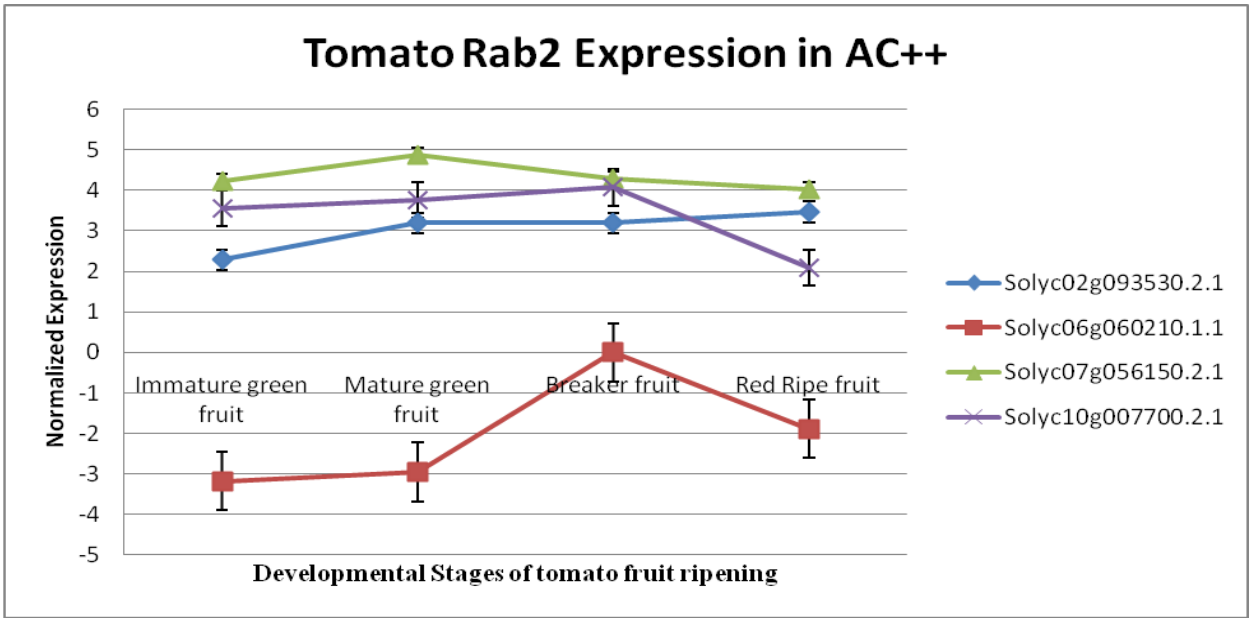


Fig.6.5 Relative expression profile of Rab2 GTPase in wild type (AC++) fruits. The figure shows the gene expression pattern of four RabB/Rab2 class of GTPases at the Immature green (IMG), Mature Green (MG), Breaker (Brk) and Fully ripe or Red ripe (RR) fruit of AC++.

The fig.6.5 shows the expression pattern of the Rab2/RabB GTPase in the wild type tomato (AC++) at the different developmental stages i.e. IMG, MG, Brk and RR. The Solyc06g060210 gene belonging to Rab2/RabD class of GTPases is down regulated at IMG and MG stages as compared to the other three group members. Its expression slightly increases only at breaker stage but then again declines like other Rab2 GTPases.

There is no significant difference in the expression pattern of the other three Rab2 GTPases with SolycIds- Solyc02g93530, Solyc07g056150 and Solyc010g007700. They follow similar expression pattern during the developmental stages right from IMG to MG. Solyc02g93530 increases a little at IMG to MG stage but there is significant change at the MG, B and RR stage. Solyc010g007700 expression remains more or less same at MG and Breaker but is significantly decreases at the red ripe fruit developmental stage as compared to the other Solyc02g93530 and Solyc07g056150 at red ripe stage.

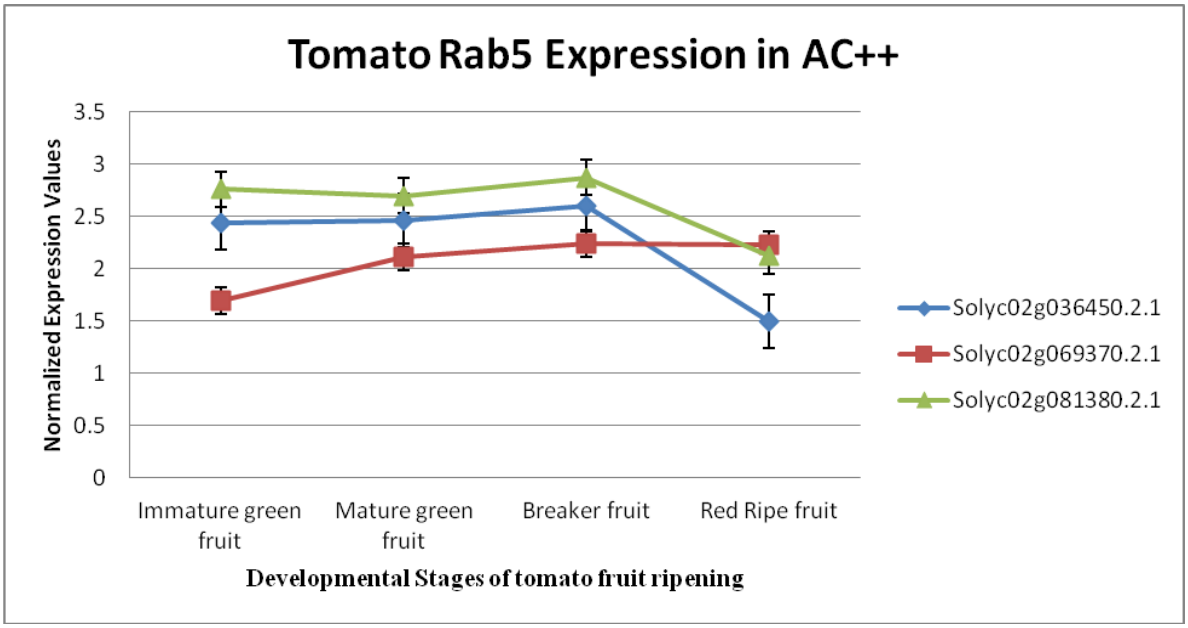


Fig.6.6 Relative expression profile of Rab5 GTPase in wild type (AC++) fruits The figure shows the gene expression pattern of three Rab5/RabF class of GTPases at the Immature green (IMG), Mature Green (MG), Breaker (Brk) and Fully ripe or Red ripe (RR) fruit of AC++.

The fig.6.6 shows the expression pattern of the three Rab5/ RabF GTPase in the wild type tomato (AC++) at the different developmental stages i.e. IMG, MG, Brk and RR. The expression of two Rab5 GTPases with the Solyc Ids Solyc02g036450 and Solyc02g081380 follow almost same pattern. Their expression is fairly similar at the IMG stage to MG stage and no significant rise from MG to Breaker stage but the expression of both genes decline at the red ripe stages.

In contrast to the above two gene of Rab5 GTPase the gene Solyc02g069370 shows altogether a gradual increase through out the fruit development right from the IMG to MG stage and from MG to Breaker stage, but there is no decline in the expression of this gene from Breaker to red ripe stage of fruits as compared to the other 2 Rab5 GTPase which decline at red ripe stage. Out of total Rab5 genes in tomato the above graph represents only three Rab5 GTPases for whose the expression data was available for wild type AC++ tomato.

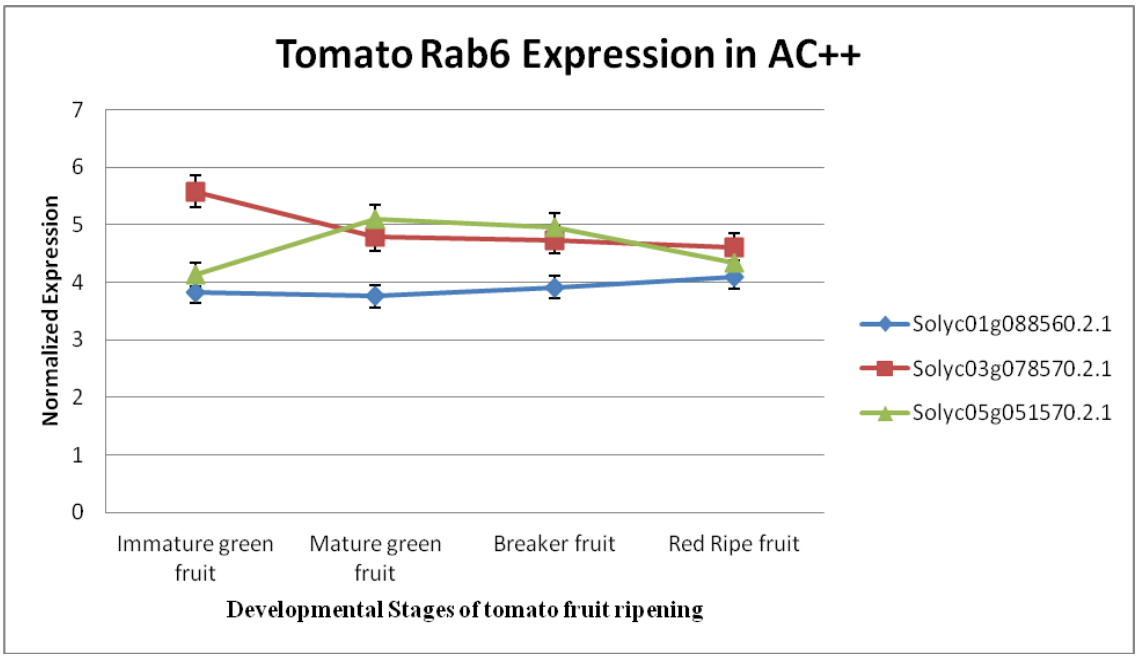


Fig.6.7 Relative expression profile of Rab6 GTPase in wild type AC++ fruits
The figure shows the gene expression pattern of three Rab6/RabH class of GTPases at the Immature green (IMG), Mature Green (MG), Breaker (Brk) and Fully ripe or Red ripe (RR) fruit of AC++.

The fig.6.7 shows the expression pattern of the three Rab6/ RabH GTPase in the wild type tomato fruit (AC++) at the different developmental stages i.e. IMG, MG, Brk and RR. The expression of Rab6 GTPases with the Solyc Ids Solyc01g088560 remains fairly stable through out the fruit developmental stages right from IMG to MG, MG to Breaker and from breaker to Red ripe stage.

The expression pattern of other Rab6 gene with Solyc03g078570 is different where its expression decreases from immature to mature green stage which is in contrast to the expression of Solyc05g051570 which increases from IMG to MG stage. The expression of both these genes doesn't show any change from MG to breaker stage. But in the case of Solyc05g051570, its expression slightly decreases from Breaker to the red ripe stage whereas the expression of the Solyc03g078570 gene remains more or less same from breaker to red ripe stage of the fruit.

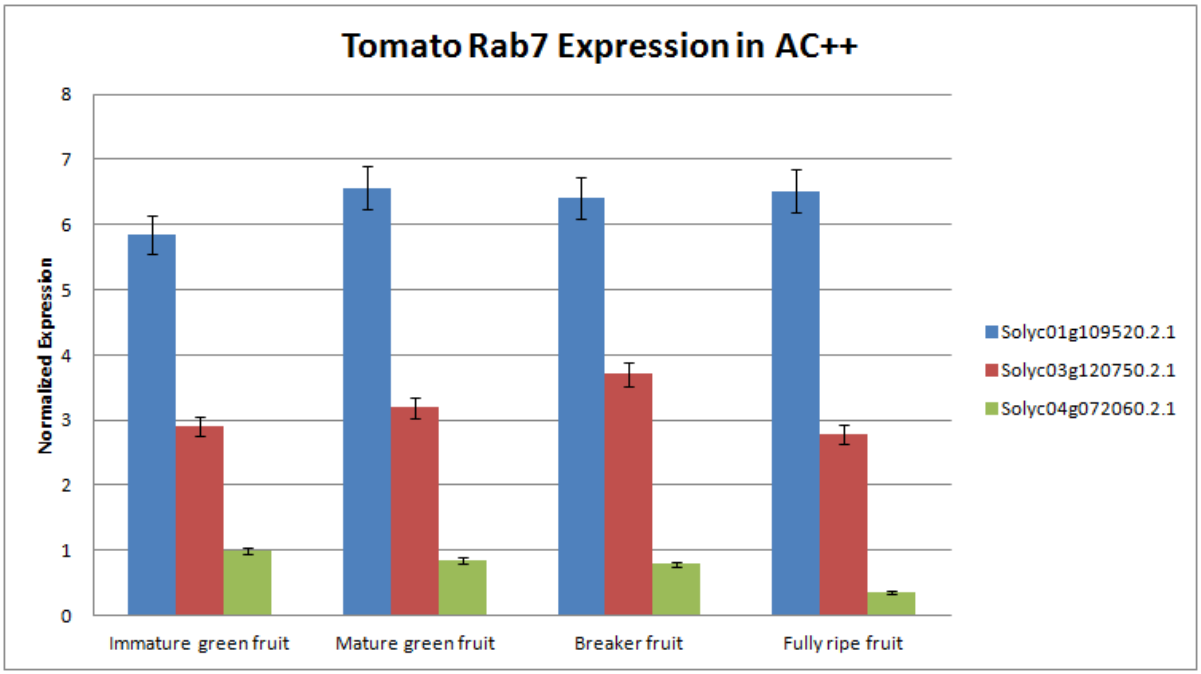


Fig.6.8 Relative expression profile of Rab7 GTPase in AC++
The figure shows the gene expression pattern of three Rab7/RabG class of GTPases at the Immature green (IMG), Mature Green (MG), Breaker (Brk) and Fully ripe or Red ripe (RR) fruit of AC++.

The fig.6.8 shows the expression pattern of the three Rab7/ RabH GTPase in the wild type tomato fruit (AC++) at the different developmental stages i.e. IMG, MG, Brk and RR. The Rab7 GTPase with the Solyc Id Solyc01g109520 and Solyc03g120750 both show gradual increase in the expression from the immature green stage to mature green stage but in case of Solyc01g109520 the expression at MG, breaker and red ripe stage doesn't change much, whereas the expression of Solyc03g120750 declines from breaker to red ripe stage.

The Rab7 GTPase gene with Solyc Id Solyc04g072060 is expressed much significantly lower as compared to Solyc01g109520 and Solyc03g120750 throughout the developmental stages.

It is evident from the graph that the expression of Solyc01g109520 gene is significantly higher as compared to the other two Rab7 GTPases Solyc04g072060 and Solyc03g120750 throughout the developmental stages.

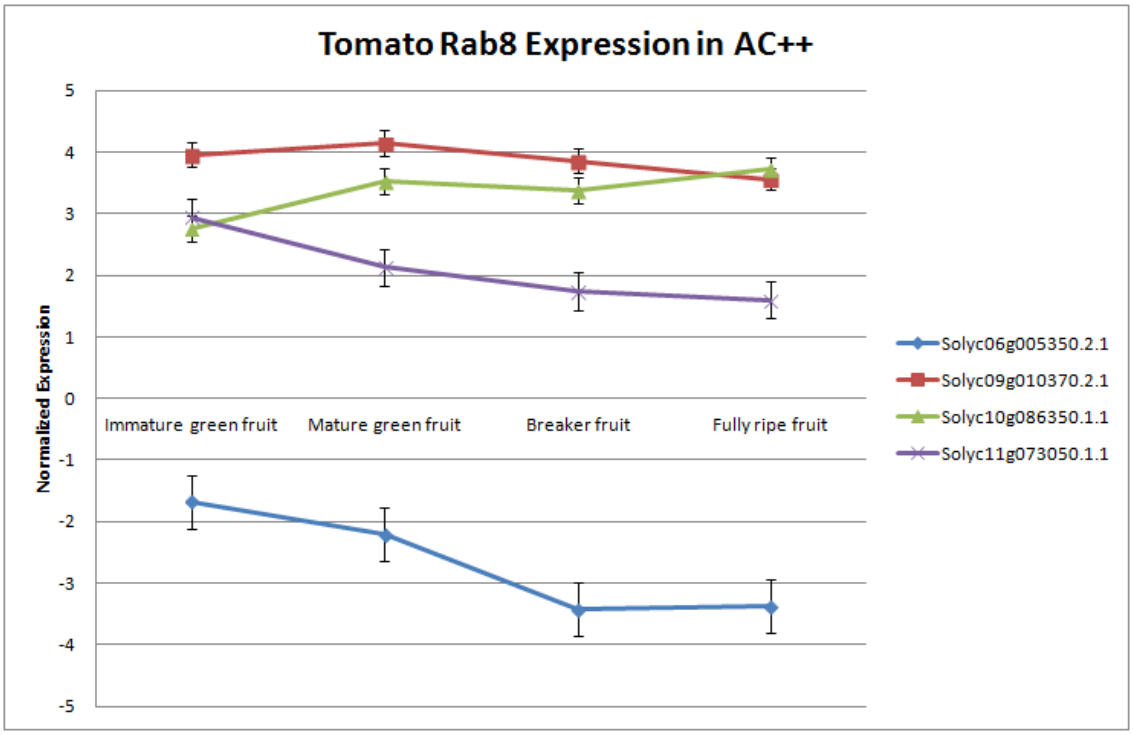


Fig.6.9 Relative expression profile of Rab8 GTPase in AC++
The figure shows the gene expression pattern of four Rab8/RabE class of GTPases at the Immature green (IMG), Mature Green (MG), Breaker (Brk) and Fully ripe or Red ripe (RR) fruit of AC++.

The fig.6.9 shows the expression pattern of the four Rab8/ RabE GTPase in the wild type tomato fruit (AC++) at the different developmental stages i.e. IMG, MG, Brk and RR. The expression of Solyc06g005350 is down regulated as compared to the other three Rab8 GTPases. Its expression decreases from IMG to MG and it rapidly decreases from mature green to breaker stage but remains stable with no significant change form the breaker to red ripe stage. The gene Solyc11g073050 also follows the same pattern of Solyc06g005350 where its expression gradually decreases from IMG to MG and a fair decline from MG to breaker stage and remains more or less similar from breaker to red ripe.

In contrast to the expression of the above two Rab8 GTPase the gene Solyc09g010370 and Solyc10g086350 show a gradual increase from immature green stage to mature green stage but the expression slightly declines from MG to breaker. The expression of both these genes is almost similar at the red ripe fruit developmental stage.

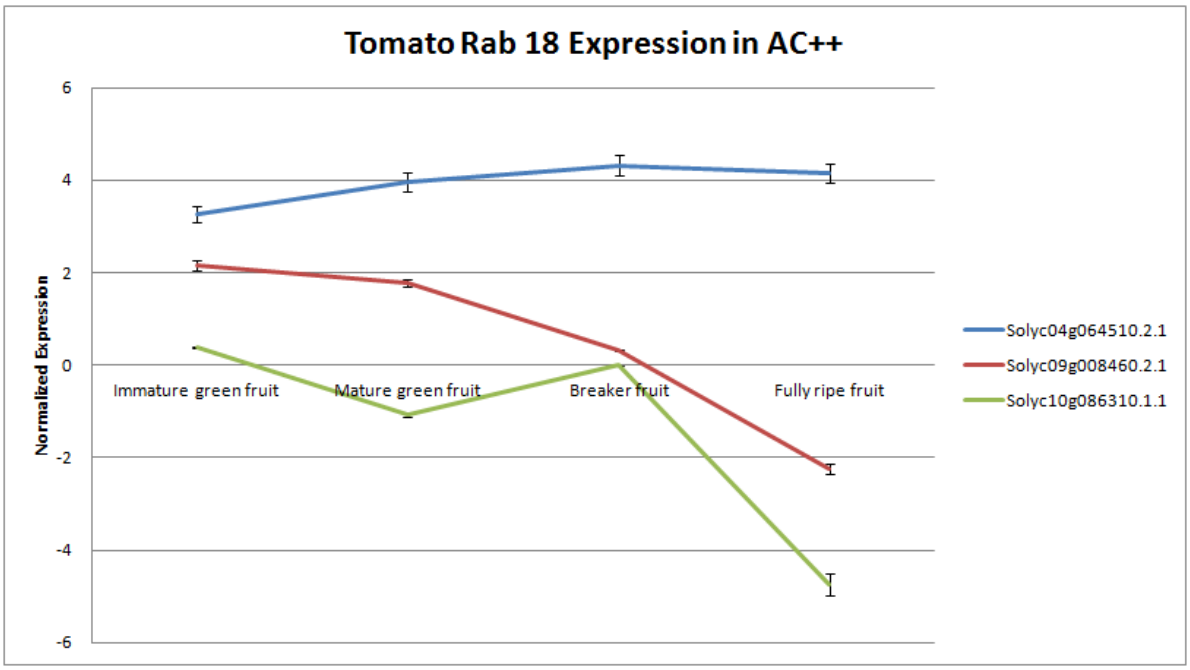


Fig.6.10 Relative expression profile of Rab18 GTPase in AC++
The figure shows the gene expression pattern of three Rab18/RabC class of GTPases at the Immature green (IMG), Mature Green (MG), Breaker (Brk) and Fully ripe or Red ripe (RR) fruit of AC++.

The fig.6.10 shows the expression pattern of the three Rab18/ RabC GTPase in the wild type tomato fruit (AC++) at the different developmental stages i.e. IMG, MG, Brk and RR.

The expression of the Rab18 gene-Solyc04g064510 is up-regulated as compared to the other two Rab18 GTPases. As the expression gradually increases from the immature green stage to mature green stage and then slightly increases to breaker stage, but does not decrease significantly at the red ripe stage.

In contrast to the expression of the above Solyc04g064510 the other two Rab18 GTPase *viz.* Solyc09g008460 and Solyc10g086310 show decrease in expression form the immature green stage to the mature green stage. Both also show same pattern of decline in expression from breaker to red ripe stage. The only difference being that the Solyc10g086310 Rab18 GTPase is significantly down regulated at the red ripe stage of fruit development as compared to the other stages of fruit development and other Rab18 GTPases likewise.

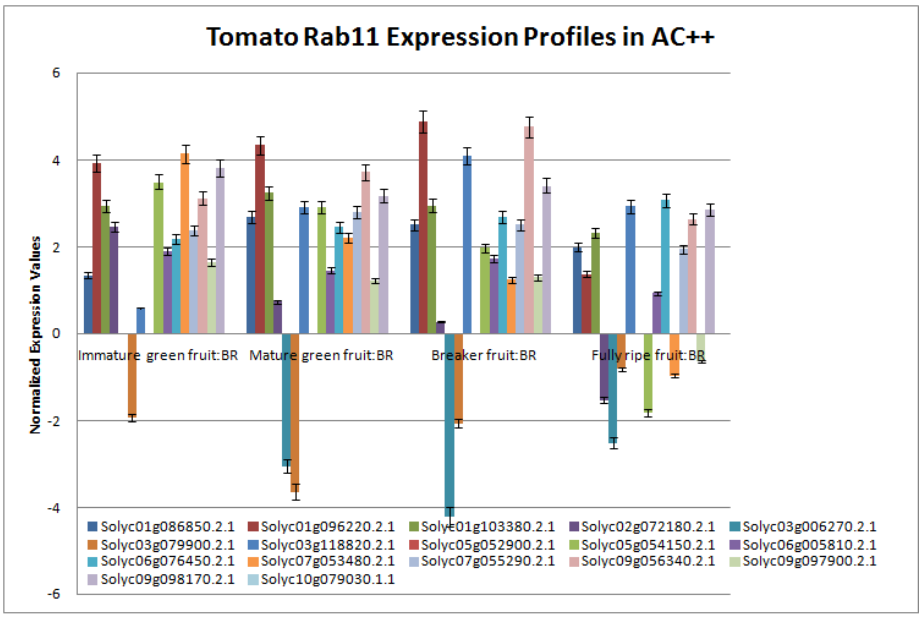


Fig.6.11 Bar Graph of Relative expression profile of Rab11 GTPase in AC++ The figure shows the gene expression pattern of Rab11/RabA class of GTPases at the Immature green (IMG), Mature Green (MG), Breaker (Brk) and fully ripe or Red ripe (RR) fruit of AC++.

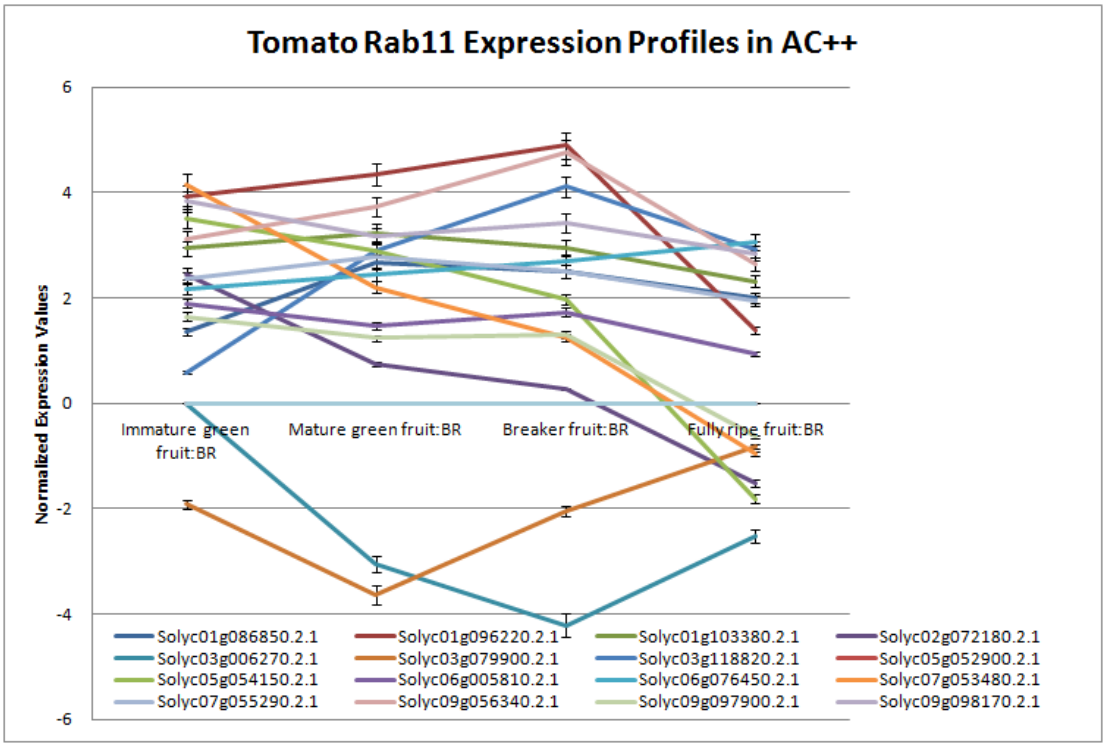


Fig.6.12 Line graph of Relative expression profile of Rab11 GTPase in AC++ The figure shows the gene expression pattern of Rab11/ RabA class of GTPases at the Immature green (IMG), Mature Green (MG), Breaker (Brk) and fully ripe or Red ripe (RR) fruit of AC++.

The fig.6.11 shows bar graph of the gene expression pattern of the 17 genes available out of total 25 annotated Rab11/ RabA GTPase in the wild type tomato fruit (AC++) at the different developmental stages i.e. IMG, MG, Brk and RR. The same data is represented in the line graph format for simplicity (Fig.6.12)

As can be seen from the bar and line graph the expression profiles was mined for the following Solyc IDs

Solyc01g086850; Solyc01g096220; Solyc01g103380; Solyc02g072180
Solyc03g006270; Solyc03g079900; Solyc03g118820; Solyc05g052900
Solyc05g054150; Solyc06g005810; Solyc06g076450; Solyc07g053480
Solyc07g055290; Solyc09g056340; Solyc09g097900; Solyc09g098170
SOlyc10g079030

The expression of the gene Solyc01g096220 increases from the IMG to MG and from MG to breaker fruits but is significantly down regulated and declines at the red ripe stage. This gene is also significantly highly expressed at the IMG, MG and breaker stages as compared to all the other RAB11/RabA GTPases.

Likewise in case of our target Rab11a GTPase with Solyc Id Solyc07g055290, its expression increases from IMG to MG and starts to decline at the red ripe stages as was observed in qRT-PCR results. This confirms the expression pattern of its higher expression in immature and green fruits and then declines from breaker to the red ripe fruits.

The down regulated genes include the Solyc03g006270 which is down regulated in the IMG, MG, breaker and red ripe. But the expression increases inversely in case of low expression. Likewise the gene Solyc03g079900 is also down regulated at the IMG, MG, breaker and red ripe stage following the same pattern of the gene Solyc03g006270.

The expression pattern for the gene Solyc02g072180 is interesting as it constantly decreases from IMG to MG; MG to breaker and its expression declines remarkably in red ripe stage where it is down regulated as compared to its own expression in the previous developmental stages. Rest of the genes follow the same pattern of being expressed at MG then having a gradual increase from IMG to MG and then decline at the red ripe stage. The Solyc09g056340 gene is highly expressed in breaker but it also declines at the red ripe stage.

Along with the data retrieval for the gene expression in wild type *Ailsa craig* (AC++), two more datasets were also analysed to find the expression pattern of Rab11 GTPase in Heinz cultivar of tomato and of *Solanum pimpenifolium.*

The fig.6.13 shows the line graph of the relative expression profile of 25 Rab11 GTPase in Tomato Heinz cultivar. The line graph shows the expression of Rab11 GTPase in Heinz at different developmental stages including flowers, flower buds, 1cm fruit, 2 cm fruit, 3cm fruit, Mature green fruit, Breaker fruit and breaker +10 days. Whereas fig.6.13 shows the bar graph for the same set of genes but only in 1cm fruit, 2 cm fruit, 3cm fruit, Breaker fruit. The following solyc IDs were used to generate the expression profiles in Heinz tomato.

Solyc01g086850; Solyc01g096220; Solyc01g103380; Solyc02g072180; Solyc03g006270; Solyc03g064020; Solyc03g079900; Solyc03g118820; Solyc04g012180; Solyc05g052900; Solyc05g054150; Solyc06g005810; Solyc06g076450; Solyc07g053480; Solyc07g055290; Solyc09g056340; Solyc09g097900; Solyc09g098170; Solyc10g008840; Solyc10g045550; Solyc10g079030; Solyc11g010100; Solyc11g012460; Solyc12g010790; Solyc12g014080.

According to the expression profiles generated in fig. 6.13 all Rab11 GTPase show an increased pattern of expression from flowers to flower buds. As shown in fig 6.13 the genes Solyc03g064020, Solyc05g052900 and Solyc10g079030 are down regulated in 1cm fruit. The genes Solyc03g006270, Solyc03g079900 and Solyc11g010100 are down regulated at breaker stage. Most of the Rab GTPases follow a pattern of increased expression from 1cm fruit to 3cm and from 3cm to breaker stage as was observed in case of AC++ for majority of Rab11 GTPases. Fig.6.14 shows the expression pattern of Rab11 GTPases for Heinz cultivar at 1cm fruit, 2 cm fruit, 3cm fruit and Breaker fruit.

The line graph in fig.6.15 shows the expression of 15 genes of Rab11 GTPase in *S. pimpenifolium* at three different developmental stages including IMG, Breaker and Breaker +5, whereas fig.6.16 shows the bar graph for the same. In most cases the expression of Rab11 GTPase gradually declines from MG to Breaker and from breaker to the B+5, with the exception of the three genes Solyc03g006270, Solyc03g079900, Solyc05g052900 with erratic pattern of expression which are also supposedly down regulated in all three stages.

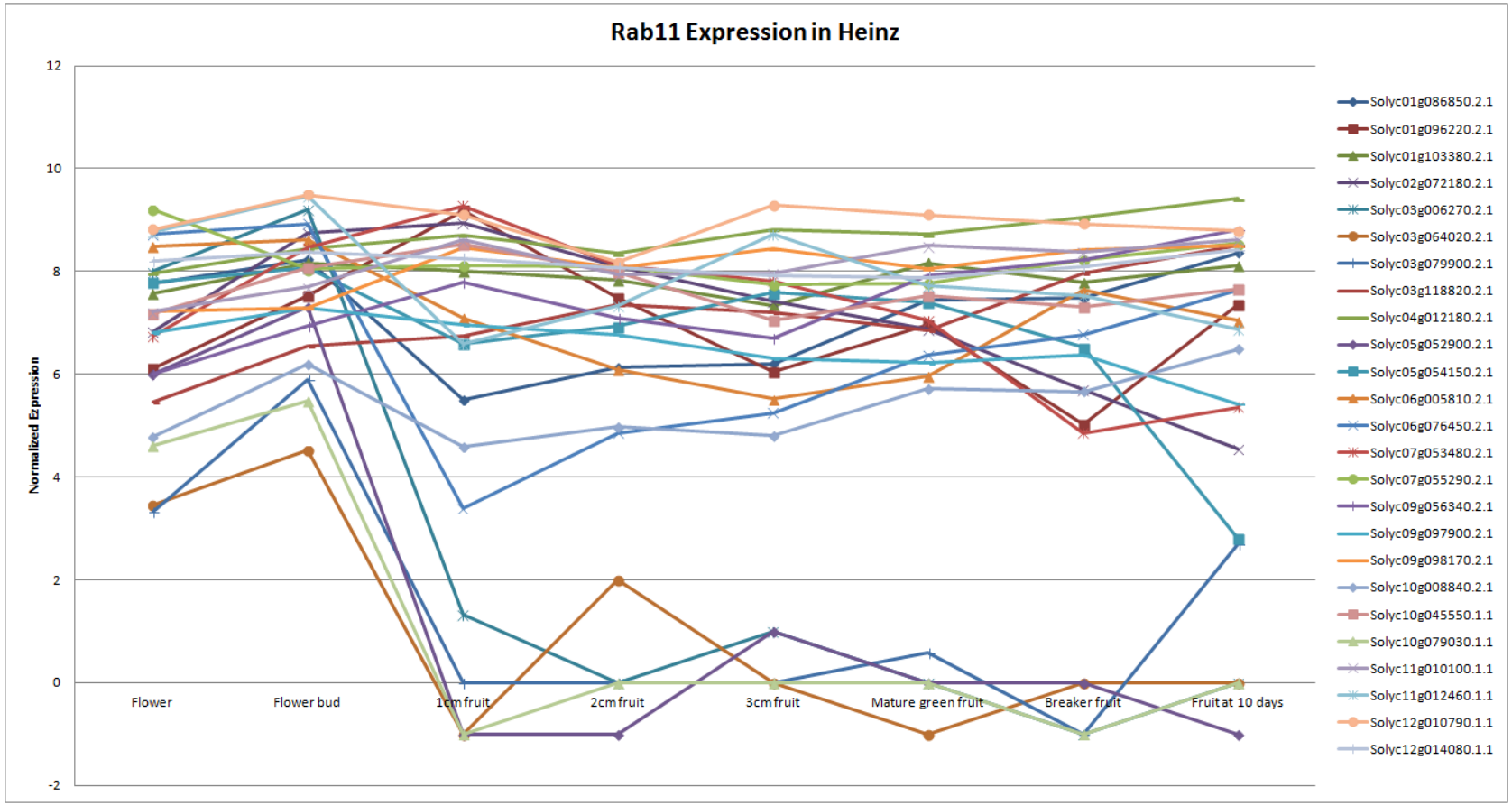


Fig.6.13 Relative expression profile of Rab11 GTPase in Tomato Heinz cultivar

The line graph shows the expression of Rab11 GTPase in Heinz at different developmental stages including flowers, flower buds, 1cm fruit, 2 cm fruit, 3cm fruit, mature green fruit, Breaker fruit and breaker +10 days.

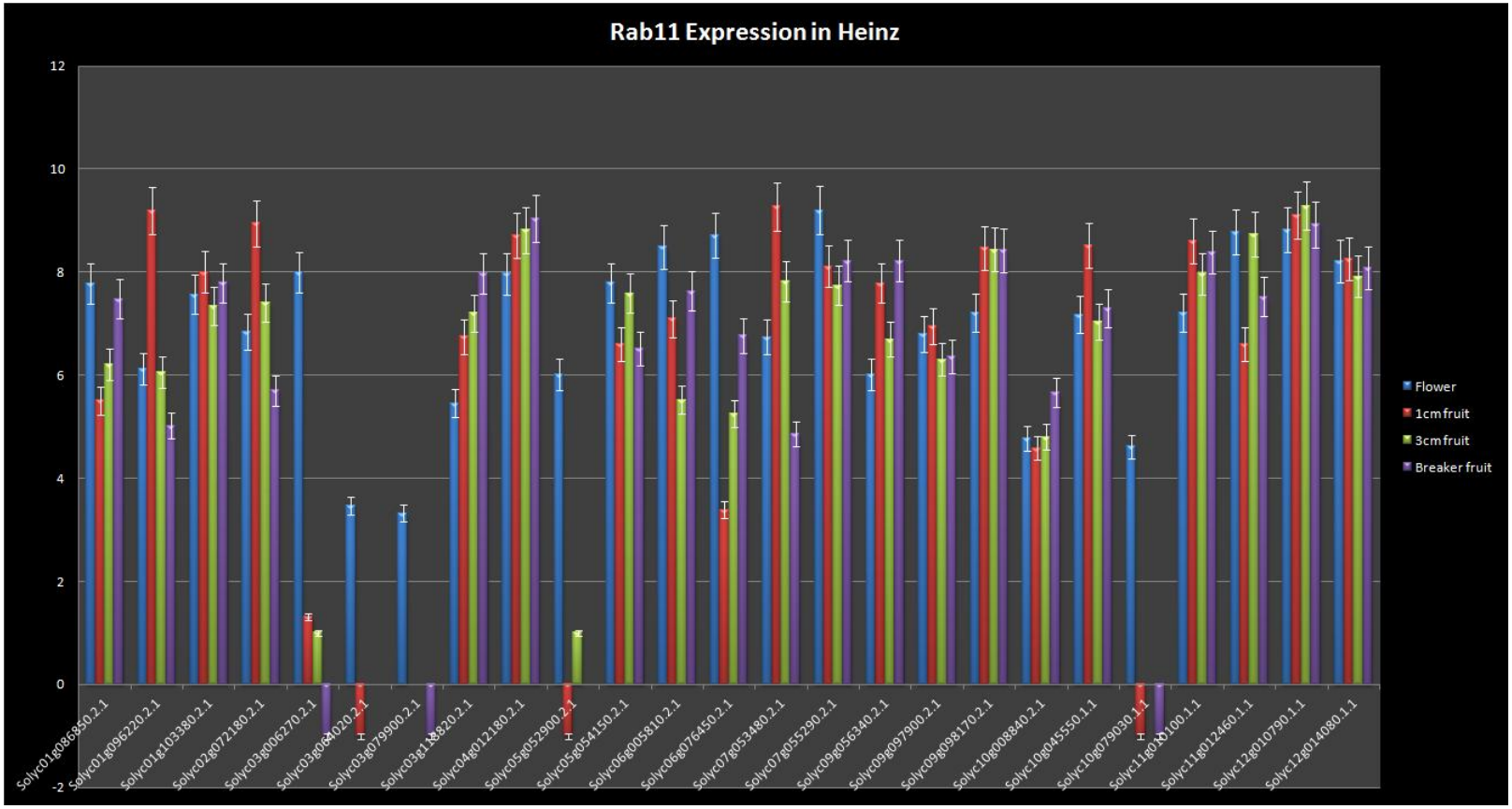


Fig.6.14 Bar Graph of Relative expression profile of Rab11 GTPase in Tomato Heinz cultivar

The bar graph shows the expression of 25 Rab11 GTPase in Heinz at four different developmental stages including 1cm fruit, 2 cm fruit, 3cm fruit and Breaker fruit.

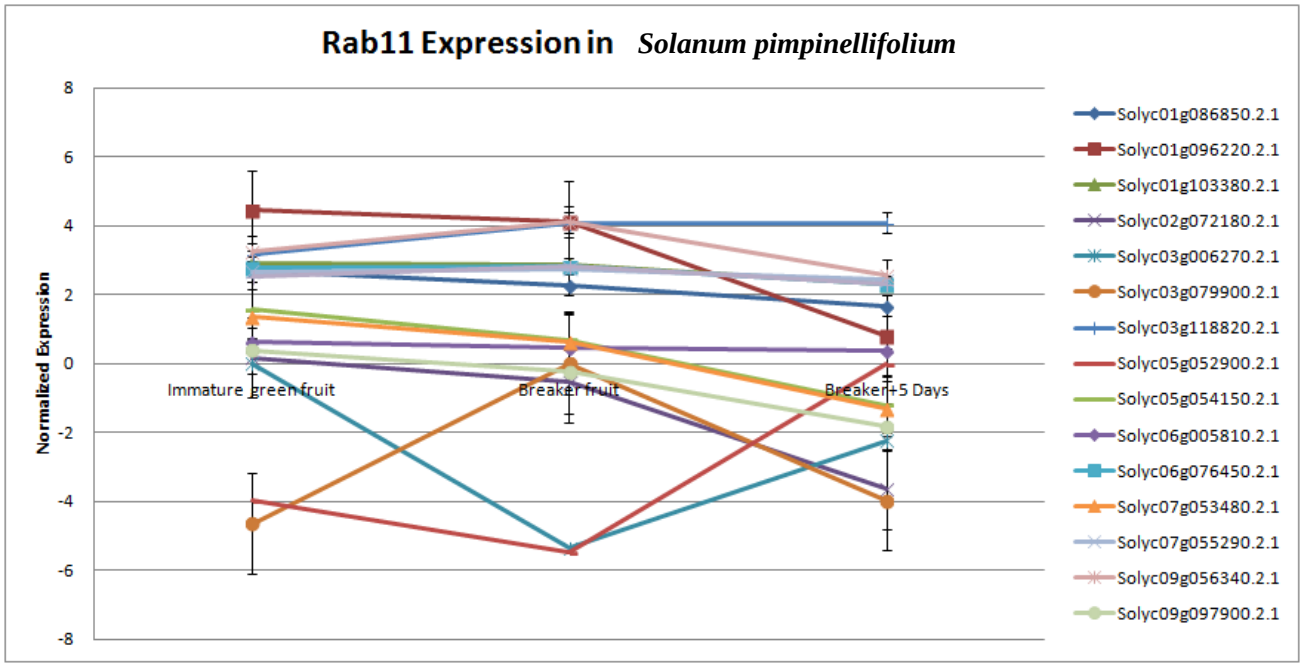


Fig.6.15 Relative expression profile of Rab11 GTPase in *Solanum pimpinellifolium*. The line graph shows the expression of 15 Rab11 GTPase in *S. pimpinellifolium*.at three different developmental stages including IMG, MG and Breaker fruit.

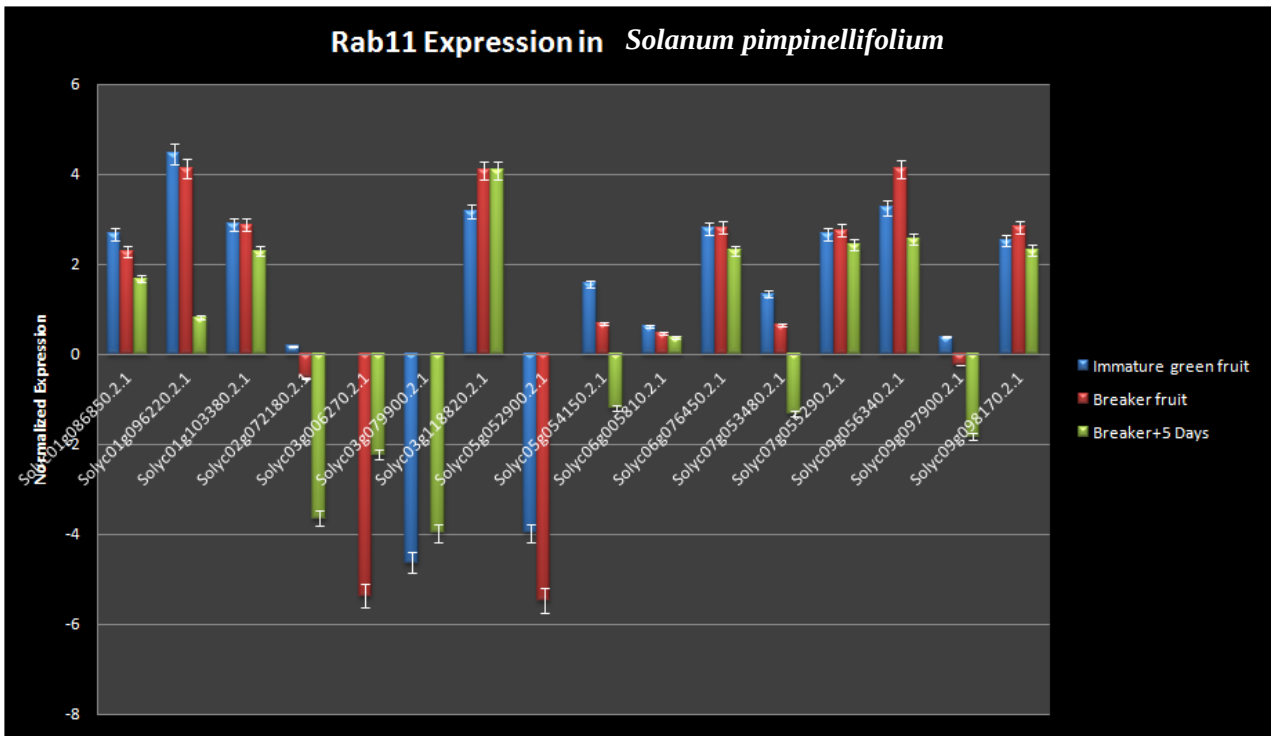


Fig.6.16 Bar Graph of Relative expression profile of Rab11 GTPase in *Solanum pimpinellifolium*. The bar graph shows the expression of 16 genes of Rab11 GTPase in *S. pimpinellifolium*. at three different developmental stages including IMG, MG and Breaker +5 fruit.

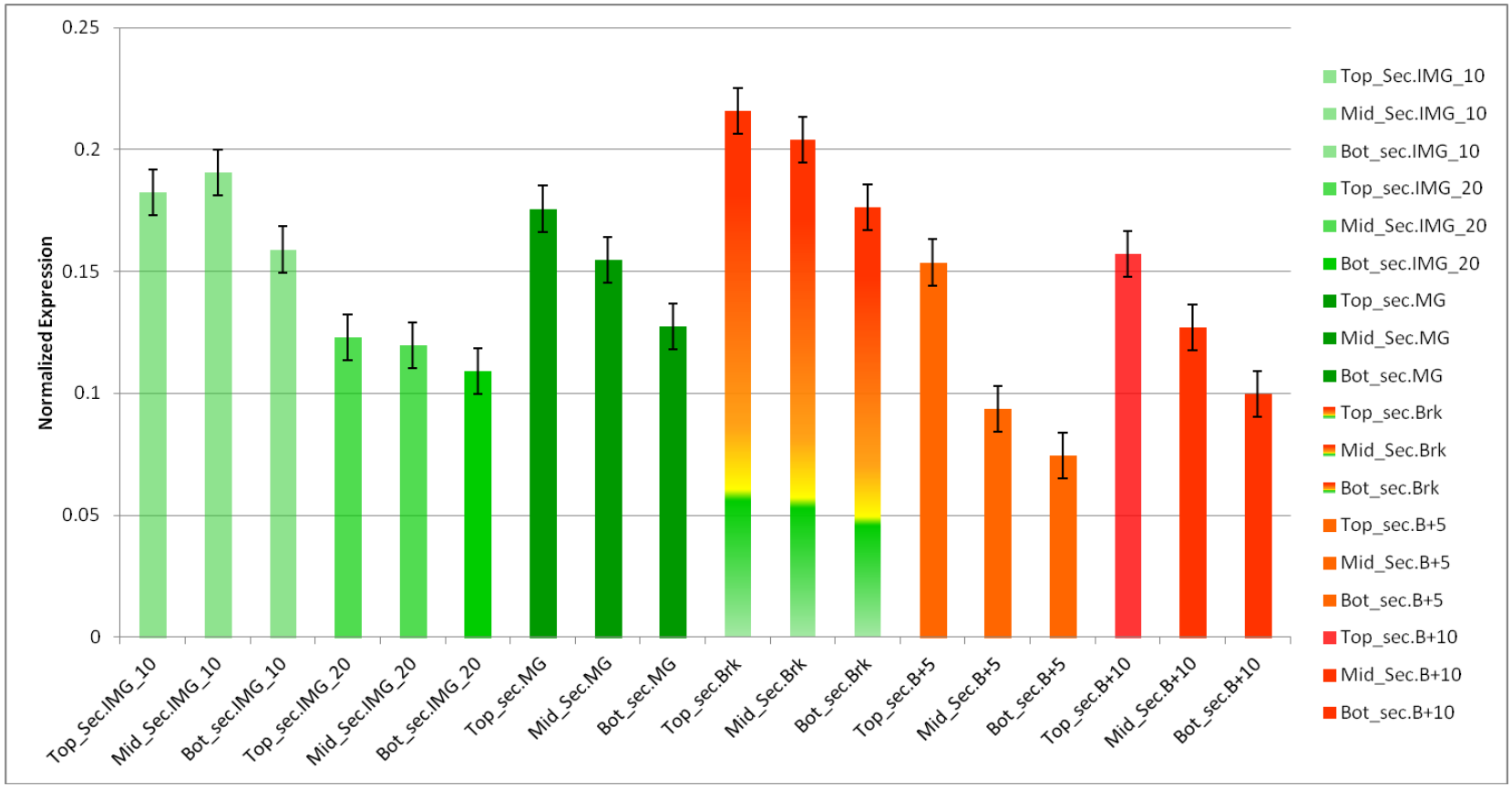


Fig.6.17 Bar Graph of Relative expression profile of LeRab11a GTPase in top, middle and bottom part of tomato fruit at IMG, MG, Breaker (Brk), B+5 and B+10. The fig. represents the different ripening stages IMG at 10DPA, IMG at 20 DPA, MG, Brk, B+5 and B+10 of the fruit's top, middle and bottom section. The Chart was prepared in excel using specialized function tabs for colouring pattern of bars according to the respective fruit ripening stages. Error bars Represent ±SE for n=3 for each sample at each respective stage.

Fig.6.17 shows the expression pattern of *LeRab11a* GTPase in top, middle and bottom part of wild type (AC++) tomato fruit at six different developmental stages. The six stages are IMG10- immature green at 10 days, IMG20-immature green at 20, MG-mature green, Brk -Breaker, B+5-breaker plus 5 days and B+10-breaker plus 10 days fruit. The three main section of fruits used for generating the expression profiles for the experiment were top, middle and bottom section of the tomato fruits. The Chart was prepared in excel using specialized function tabs for colouring pattern of bars according to the respective fruit ripening stages. The raw data was obtained from SGN and the data was normalized to generate the expression profiles for *LeRab11a* GTPase gene only.

As sown in the graph (fig.6.17) the expression pattern between the three section *i.e.* top, middle and bottom part of fruit is similar at all the stages where in the expression is higher in case of top section of the fruits as compared to the middle and bottom section of the fruits in all the developmental stages highlighted. The expression of *LeRab11a* GTPase gradually increases from IMG to MG up to breaker stage, and then declines at B+5 and B+10 stages. The comparatively higher expression in the top section of tomato fruit is reasonable since the top section of the tomato fruit is attached to the calyx and plant, and the fruit sources all of its nutrients from the top section. The change in colour at breaker and post breaker stage starts from the bottom section of the fruits. The higher expression in top section may well elucidate the necessity of the Rab11a GTPase to traffic more proteins during the cell division and cell expansion phase. It would be interesting to generate expression profiles or different GTPase for the individual sections of tomato mesocarp and endocarp, layers of pericarp, as well as the cuticle and columella to find for the differences.

6.5 Conclusion

The primers were designed for the quantitative real time PCR for the selected *Le Rab11a* GTPase using PRIMER 3 software from the SGN website. The primer designing was difficult since the group of *Rab11 GTPase* gene share high similarity and homology. The exhaustive BLAST searches were carried out in TIGR DFCI TGI database. The alignments of primer pairs with the respective gene sequences were generated to ensure primers hit and amplify the target

sequence only. The digital PCR or *in silico* PCR was also used to confirm and to correlate the TC with SGN-Unigene ids and if possible to assign the available Solyc-Ids to the genes. This was difficult as discussed in this chapter, but an alternative of reverse blast to hunt the SGN-Unigene IDs was employed taking into account the previously compiled and designed database of TIGR TC and SGN-Unigene ids. To date there are many Solyc Ids with no associated Unigenes, since the annotations are assigned automatically by scripts and not manually curated. Thus the reverse blast was able to associate the SGN-Unigene to the corresponding Solyc Ids. Thus primers were validated using three different approaches, in three types of databases and finally with the run of RT PCR showing unique expected size bands. The results from the qRT PCR indicates that the three major genes viz., TC199565, TC208692 and TC202127 which are up regulated in wild type during the early developmental stages and down regulated during the later developmental stages which can act as the putative candidate genes for further studies. Their expression was low as compared to the transgenic fruits at different developmental stages. The expression profile of the target Rab11 GTPases was thoroughly generated and analysed where it showed higher expression in the immature and mature green expanding fruit and the decline in expression in the post breaker and red ripe stages. The expression profiles were also generated by analysing the data available from the different database in TED and SGN. Apart from Rab11 GTPases the expression profiles for Rab1, 2, 5, 6, 7, 8 and Rab18 in Ailsa Craig (AC++) were generated which led to the identification of putative candidate genes with differential expression pattern at different developmental stages. The expression profiles of Rab11 GTPases were also explored in Heinz and *S. pimpinellifolium.*

In silico analysis and classification of Rab GTPases of tomato

A Rab11 GTPase was isolated from a cDNA library from mango by Zainal *et al*., in 1996 which was expressed in ripening related manner. Lu *et al*., (2001) reported that a cDNA clone from the tomato fruit encoding a protein homologous to *rab11/YPT3* was developmentally regulated during fruit ripening. The antisense fruit changed colour but failed to soften normally. Different *Rab11* GTPase genes have been isolated from plants including pea, lotus, rice, soybean and *Arabidopsis*. Rab11 GTPases are involved in the trafficking of vesicles from TGN to PM, thus it plays an important role in plants for trafficking of proteins important for the cell wall modification in different tissues of plant (Lycett, 2008).

The first objective of the present study was to find the total number of Rab GTPases in tomato by *in silico* analysis (Chapter 3). In order to find tomato Rab GTPases using the bioinformatic tools and software packages, the information about earlier reported Rab GTPases was mined and analyzed. Using the TAIR database of *Arabidopsis*, a total of 57 Rab GTPases were mined and used to screen the TIGR-DFCI Tomato Gene Index database.

Protein Structure Prediction for LeRab11a GTPase

The protein sequence of the LeRab11a (Q9SCB1) was used for the Interpro-scan. The results revealed that it contained the Ras GTPase family (IPRO01806), Ras small GTPase Rab type (IPRO03579), small GTP binding domain (IPRO05225), domains depicting it to be a member of the Ras superfamily and was a confirmed Rab GTPase (fig.3.2). Analysis of LeRab11a GTPase secondary structure prediction (fig.3.3) by MUSTER led to the identification of the helix, sheets and coils of the protein. The secondary structure helps to identify the folding of the proteins based on the helix, sheets and coils which represent the catalytic and interacting domains which retains the proper functionality of the protein. For a more comprehensive and in-depth analysis of LeRab11a GTPase, the Phyre2 server (Kelley *et al*., 2015) was used to pull down the domain architecture which gave more clear results about the individual domains (fig.3.4). The phyre2 analysis showed the GTP/Mg^{2+} binding site, GEF and GDI interaction sites, the characteristic Switch-I and II region, G (G1-G5) domains, Rab Family (RabF1-5)

and Rab subfamily (RabSF1-4) motif of the LeRab11a GTPase. The results are in agreement with the classification as described by Pereira-Leal and Seabra (2000). The 3D structure was generated for LeRab11a by using '2f9ma' as reference for 3D modelling by the Ribbon software and Swiss modeller with 100% confidence by single high scoring template '2f9ma' (fig. 3.5). The structures of at least 16 different Rab proteins in either their active (GTP-bound) or inactive (GDP-bound) state have been solved and are available online (Grosshans *et al*., 2006; Lee and Mishra, 2009). As of February 2014, 555 structures of Ras-superfamily proteins have been deposited in the protein databank (PDB), either in un-complexed form or bound to effectors or other regulatory proteins. The 751 sequences contain either GTP or a GTP analogue (431) and GDP (320) respectively. Diez *et al*., (2011) measured the complexity in the number of genes of the canonical Ras switch genetic system (including Ras, RasGEF, RasGAP and RapGAP families) from 24 eukaryotic organisms and correlated it with their genome size which was found to be inversely correlated to their evolutionary distances from humans. These reports put the present study on tomato in perspective of plants where apart from *Arabidopsis* very few plant species have been fully analysed to identify and classify all the Rab GTPases with their signature motifs and domain.

Sequence Analysis and Phylogeny-TIGR DFCI TGI Database

Large scale 1:1 and 1: many (multiple sequence BLAST) BLAST searches were used to screen the tomato gene index (TGI) database to hunt for the corresponding TC-Ids of tomato Rab GTPase. The E-values, score hits, percent identity and percent similarities, amino acid sequence of the polypeptide, and available annotation were taken into consideration for finalizing the numbers and classes of tomato Rab GTPase (see supplemental files for BLAST hit results for TIGR Database). Table 3.9 enlists the total number of Rab GTPase found in tomato from the TIGR database based on the release 11 and 12 with their role in intracellular vesicle trafficking between different cellular compartments as described by Lycett (2008). The TIGR database was updated three times (Release 11, 12 and Release13), and for every single update all the searches were repeated to update the current tomato Rab GTPase database.

Precautions while analysing, annotating and setting up the nomenclature for the gene family in question.

1. The term homologous is used for genes that share a common evolutionary origin. The sequences may be homologous or not, and the term 'similar' should be used instead of partially or highly homologous. The term paralogous is used for genes that originated by gene duplication, whereas the term orthologous is used when the genes originated by speciation from common ancestor and may have a newly evolved function. In the present study *Arabidopsis* Rab GTPase AtRabA and tomato Le/SolycRabA were designated as orthologs and within RabA e.g. AtRabA1, A2 or Le/Solyc RabA1, A2 were designated as paralogs. The cases where the orthologous or paralogous relationship is not easy to establish through phylogeny, then the term putative ortholog should be used as was observed in case of tomato ARFs and ARLs. Homeologues term should be used for the genes in case of the polyploid species e.g. wheat (hexaploid) or potato (tetraploid).
2. The annotation and the specific nomenclature should be clear while analysing and annotating the new sets of genes and gene families in an organism. Initially tomato Rabs were designated as LeRabs since the scientific name of tomato prevalent during that time was *Lycopersicum esculentum* and later on the current accepted scientific name of *Solanum lycopersicum* was used e.g. SolycRabs. The preferred method adopted by the commission on plant gene nomenclature (CPGN) is to prefix the initials of the scientific name of the organism before the gene name ***e.g. Arabidopsis thaliana*** Rabs as AtRab, in rice (*Oryza sativa*) as OsRab, or in tomato (*Solanum lycopersicum*) Rabs as SlRab or Solyc Rabs. For the gene family with sub family members the names should be carefully assigned based on the phylogeny and exact sequence similarities with other references e.g. AtRabA1, AtRabA2 and likewise in tomato as Solyc RabA1 and Solyc RabA2.
3. One of the problems with sequence analysis is that many sequences have been annotated in an automated manner without manual curation. This results in annotations which cannot be transferred. The major drawback is the annotation errors due to automation can be further propagated and may lead to erroneous predictions as highlighted by Brenner, 1999, Koski and Golding, 2001 and Gilks *et al*., 2005. This was also observed in the present study while analysing

the SGN database wherein some of the sequences were annotated as Rab5/F and Rab18/C which on further in depth analysis turned out to be septum promoting GTPases as discussed later in this chapter.

4. Another problem arises because of the sequence fragments or misassembled chimeric sequences in the database (e.g. EST Databases), as was observed while using the TIGR DFCI-TGI EST database in the present investigation, where duplicated genes were replicated with different TC-ids. This actually leads to same gene having two different ids. This can be rooted out only through the manual curation of data by crosschecking the individual sequences and their similarities. Thus, the solution is to use a manually curated set of Rabs against which the candidate sequences can be obtained by BLAST and then transferring the annotation from the closest hit after careful inspection.
5. It is important to note that there are several genes with same name but are altogether very different in form, function and localization. To avoid confusion it is important to consider the exact name and nomenclature while screening and mining the database for the target genes and annotation. For example in the case of RAB and ARF GTPase if one goes by the abbreviation of Rab it will fall in 2 groups i.e. *Rab* in terms of Rab GTPases and *Rab* gene with respect to ABA is referred to as RAB-'Responsive to Abscisic acid' (ABA). In case of ARF GTPase, ARF in terms of GTPases stands for 'ADP Ribosylation Factor' (ARF), whereas in terms of Auxin, ARF stands for 'Auxin Responsive Factors', in case of SAR one is for SAR GTPase and the other SAR refers to 'Systemic Acquired Resistance' protein, likewise in the case of COP where COP stands for 'coat protein' (COP) and other COP is for 'Constitutive Photomorphogenic'. These examples highlight the case where the sequence fetching is totally based on the keyword searches in the available databases where the annotations are available. Thus apart from the cognate names the unique standard gene-Ids (genebank) or protein-Ids (IPR) are much more helpful in distinguishing the result obtained, as compared to the cases of automated computer generated scripts/programs used to fetch bulk data from the databases solely based on gene/protein names or automated annotations.
6. Another type of problem encountered is that sequences which are not Rabs, but are closely related like, e.g. most problematically Rab-like (Rab-L) proteins just by chance may have a best BLAST hit that is a Rab and would therefore be

annotated automatically as being Rabs. Likewise in case of ARF and ARL GTPases it is of utmost importance to exercise caution as both families are very closely linked and associated and turn up to be the parallel hits in BLAST results where a particular ARF might be absent from the species and thus may give the best hit as the available ARL based solely on sequence similarity. This problem was encountered while analysing the data for ARF and ARL GTPases in TIGR DFCI-TGI database where large number of ARF and ARLs were initially found as compared to the newly updated SGN database (ITAG2.4). This problem can be solved by using a second database of sequences that should explicitly be excluded, including overall very close sequences like RABL, RAXs or ARLs in case of ARFs. Different domain architectures within the same orthologous groups can be interpreted as gain, loss or swap of functionality of some genes (Sjolander *et al*., 2011).

7. The major flaws observed while conducting the present investigation was erroneous reports of the parallel gene family investigation in other organisms (specific to other plants/species) where the proper nomenclature was not followed. This should be avoided as it creates confusion and may lead to inaccuracies for others trying to follow up the research in that plant with the same gene families or gene family members. New gene symbols should be compliant with the naming conventions of the relevant research community. Thus authentic use of proper nomenclature and accurate use of gene/protein symbols is a must. For example, standard information is available in TAIR database about the use of proper nomenclature and naming conventions for the scientific community (TAIR Database: https://www.arabidopsis.org/ and http://www.arabidopsis.org/portals/nomenclature/index.jsp) as well as the commission on plant gene nomenclature (CPGN) also prescribes the guidelines for naming convention. Similar efforts have been put in practice for the sequenced genomes e.g. *Medicago trunculata.*
8. The other problem encountered during the present investigation was during the bulk data retrieval. The available online databases have the options and tools to fetch bulk data for the nucleotide or protein sequences, provided the gene name or symbol is available. During bulk data retrieval from the TIGR and SGN database it was observed that the text files with sequences had many missing amino acids or nucleotides. Precautions should be exercised while using bulk

data files for generating MSA or phylogeny, as it propagates error in classification and each individual sequence should be manually/visually inspected before using them for analysis.

Previous phylogenetic analyses (Schwartz *et al*. 2007) divided the Rab family into eight functional groups (Pereira-Leal and Seabra, 2001), while later studies classified it into nine groups (Stenmark 2009) and recently published analyses using large-scale comparative genomics comparisons including hundreds of genomes divides the family into six major groups (Klopper *et al*. 2012). This stands true in case of mammalian system and other eukaryotes including the last eukaryotic common ancestors (LECA). But as far as plants are concerned in the groups of Angiosperms, the classification of Rutherford and Moore (2002) was followed for the present study as a reference for the MSA and the phyletic analysis.

The following set of simple and complex methods (programs) were used in the present study for the protein sequence analysis. The simple program uses only single algorithm to decipher if a particular set of features is present in the protein, whereas the complex ones involve more than one algorithm e.g., TMHMM (Krogh *et al*., 2001), SUPERFAMILY (De Lima Morais *et al*., 2011), Panther (Mi *et al*., 2012) and InterProScan5 (Jones *et al*., 2014).

HMMs enable classification of novel sequences to protein families and subfamilies. An automated pipeline for phylogenomic classification of proteins using the SCI-PHY (Subfamily Classification in Phylogenomics) algorithm has been used previously for the automatic subfamily identification, followed by subfamily hidden Markov model (HMM) construction (Brown *et al*., 2007). Although the results of HMM and THMM are able to generate protein profiles in questions but it largely depends on the query and proteins used. It may also differ across kingdoms and species depending on the nature of protein evolution, this can be handled well by employing the cross comparison among different plant and animal species with already established profiles of a particular protein family. In case of plants very few reports are available for the complete classification of Rab and other GTPases in plants e.g. Arabidopsis, peach, cotton. In the present investigation classification of small GTPases and their regulators was largely done by cross comparison with Arabidopsis. The complete and validated classification of different families of small GTPases and their regulators in the present

investigation was undertaken as compared to the available earlier reports of individual single group of GTPases which sets this study apart from previously published reports. After employing the above mentioned approaches in the present investigation the genes were grouped into 8 different functional classes ranging from Rab1, 2, 5, 6, 7, 8, 11 and Rab18. The protein sequence files (*.txt & *. fasta files) of all tomato and *Arabidopsis* Rab GTPase are in supplemental files in a CD. The next step was to assess and analyze the protein sequences of Rab GTPases.

Multiple sequence alignment (MSA) method enables the comparison of DNA, RNA or protein sequences in question. Several packages and softwares are available for generating MSA. The generation of accurate MSA is a key step in phylogenetic analyses. Most of the methods work well when the sequence similarity is high but very divergent or incomplete sequences are difficult to align and the MSAs often contain errors (Kemena and Notredame, 2009). The most widely used is ClustalW which is in command line interface and ClustalX2 which is GUI based. Bioedit (current Version 7.2.5, Hall, 1999) is a MSA editor software package with multiple utilities to align and edit MSA, calculate consensus, as well as for calculating the phylogenetic trees from the respective MSA. For the present study Bioedit, Jalview ver.2.8.2 (Waterhouse *et al.*, 2009) and MEGA6 were used for the comprehensive multiple sequence analysis at each step of update of TIGR and SGN database. The downside of the increased accuracy of these new methods is their high computational demands. In the case of Ras superfamily, building trees from a starting alignment of more than 900 divergent sequences is unfeasible, even for large supercomputers (Ernst *et al*. 2011). A similar problem was faced while generating a phylogenetic tree by using maximum likelihood method even for 117 sequences in the present study, as it was time intensive and took many hours. The alignment files of tomato (Tomato Rab GTPase MSA) and tomato v/s *Arabidopsis* (TAIR) are in the supplemental files (*.aln files and *.fasta alignment files, *.dnd and *.phb are tree files and bootstrapped tree files) in CD. The results from the TIGR DFCI TGI were analysed through 11, 12 and the 13th Release and from SGN database for SGN-Unigene Ids build one and two (Chapter3).

The MSA of protein sequences of Rab GTPases led to the identification of conserved amino acid sequences and the signature motifs named Rab family specific motifs (RabFs: F1–F5), that distinguishes Rabs from other members of the Ras superfamily . The individual features also support the conserved Rab family and subfamily specific domains and motifs wherein RabF1 (IGVDF), RabF2 (KLQWI), RabF3 (RF*R*S*IT), RabF4 (YYRGA) and RabF5 (LVY*DIT) were present and were evident from the multiple sequence alignments in the present study and commensurate with those reported by Pereira-Leal and Seabra (2000) and Rutherford and Moore (2002). The RabF and Rab SF motifs were present in the different classes of Rab GTPases in tomato as was evident from the MSA of individual group.

The majority of Ras and Rho family proteins terminate with a C-terminal CAAX (C=Cys, A=aliphatic, X=any amino acid) sequence (Cox and Der, 2002). The CAAX motif is the recognition sequence for farnesyltransferase and geranylgeranyltransferase-I or II. Members of the ARF family are modified at their N-termini by a myristate fatty acid. These different C termini were observed in individual groups of Rab GTPases and are characteristic of the groups which direct their localization to different endomembranes. Most of the Rab GTPases groups in tomato satisfied the above characteristic motifs apart from one protein sequence (Solyc11g008430) in the class of Rab5/F GTPase group which had an unusual elongated N-terminus which was also observed from the MSA. On comparison with the corresponding *Arabidopsis* Rab5/F GTPases (At3g54840) similar sequence was also present and this was an interesting find since most of the Rab GTPases are geranygernaylated except this one which was indicative of being myristoylated. It would be interesting to characterize this tomato Rab5/F individually to find its interaction, mode of post translation modification and the exact sub-cellular localization in the cells.

The preliminary analysis of TGI with Release12 revealed a total of 60 Rab GTPase in tomato (Table 3.9). A total of nine Rab1/D, four Rab2/B, five Rab5/F, five Rab6/H, Seven Rab7/G, eight Rab8/E, nineteen Rab11/A and three Rab18/C were found and grouped (TIGR DFCI TGI-Release12). In case of Rab1/D the N-termini had ‘MNPEYD’, Rab 18/C ‘MESS’, Rab2/B-‘MSYAY’, Rab5/F-‘MAATMAH’, Rab6/H-‘MAAV, Rab7/G-‘MSRRT’, Rab8/E-‘MAAPPA’ and

Rab11/A-'MAYRAD'. In case of Rab1/D, TC199235 which had only142 amino acid length and TC 217052 had 205 aa length with unusual inserts but still qualified for the GTPase since it had all the RabF and SF motifs and domains. Rab5/F had one member 'TC199588' with an extended N-terminus which was indicative of it being myristoylated. Among the Rab11/A the RabA1 group had a consensus N-termini of 'MGAYRA'. In case of Rab1/D the C-termini had 'MNPEYD', Rab 18/C 'MESS', Rab2/B-'GGGGCCT', Rab5/F-'SCCS', Rab6/H-'GGGCAC', Rab7/G-'GGCEC', Rab8/E-'CCGS' and Rab11/A-'GCCSS'. The comparative MSA of tomato with arabidopsis Rab GTPases also confirmed the above stated unique features and motifs. These motifs play an important role in the anchoring and targeting of the proteins to different endo-membrane compartments and play a vital role in their sub cellular localization. It would be interesting to see if mutation in these motifs can lead to mis-localization or defective membrane trafficking.

The TIGR TGI database was updated in April 2010 (Release 13). A total of 59 Rab GTPases grouped into nine Rab1, four Rab2, six Rab5, five Rab6, four Rab7, nine Rab8, nineteen Rab11 and three Rab18 were found. Out of 21 Rab11/A GTPases found, two (TC239355 and TC224630) were excluded because of incomplete sequence. A comparison of release 12 and 13 yielded almost similar results except few sequences and TC Ids which were found to be in duplicates were eliminated as discussed earlier. This highlighted the same problem with using the EST databases but careful curation was employed to finalise the total Rab GTPases in tomato. In case of *Arabidopsis* there are 26 members of RAB11/RABA (Rutherford and Moore, 2002) but in case of tomato 21 were found but 2 were eliminated because of incomplete sequences, resulting in a total of 19 Rab11 in tomato (TIGR database). These may have diverse function according to the individual plant system and may vary from tissue to tissue. The results show that each individual identified tomato Rab GTPases fall in the same cluster/clade of *Arabidopsis* Rab GTPase further validating the results.

The next step was to screen the SGN database for the corresponding SGN-Unigene ids of tomato Rab GTPases. A list of all tomato Rab GTPases of individual groups with TC Ids from TIGR release 11 and 12 were compared to the SGN-Unigene IDs. The discrepancies were found in both databases which were

eventually traced to the different time of updates of the relevant database based on curation of the old set of data. The cross comparison was able to resolve this loop hole and was able to establish the validity and total number of Rab GTPases in tomato. Once the SGN-Unigenes were identified, the information was used to pull down the probe-ids from tomato expression (TED) database to obtain the digital expression in different tissues of tomato at different stages of fruit ripening. The expression of tomato Rab11a GTPase (fig.3.14) was generated which shows the expression levels at different days of fruit development in wild type (WT), *Nr*, *Rin*, and *hp1* mutants. It is down-regulated at later stages of fruit ripening as compared to the initial stages, as was observed in the lab experiments and discussed later. The heat maps were generated encompassing the different fruit developmental stages using either Hierarchical or K-means clustering (results of individual clustering are shown in appendix). The top highly expressed individual Rab GTPases in tomato were pooled to generate the heat maps from 7 DAP to 57 DAP. The developmental stages of 7 to 57 DAP was chosen which encompasses the entire tomato fruit developmental stages right from 7 day after pollination until the late red ripe stages. The heat map shows the differential expression pattern of selected and up-regulated GTPases with one Rab18/C, two Rab2/B, two Rab5/F, one Rab6/H two Rab7/G and Rab8/E and five of Rab11/A GTPases. The differential expression pattern observed in the clustering highlights the possible role played by the individual Rab GTPases family members during the fruit development, ripening and softening. These results gave a fair idea about the key candidate genes which can be used in further studies to decipher the role of these Rab GTPases in fruit ripening and softening.

After completing analysis of Rab GTPases, other small GTPases were also analysed and identified using the same method. In tomato 13 ARF GTPase were identified as compared to 12 in *Arabidopsis*. Likewise, 18 putative tomato ARL GTPases were found as compared to 6 in *Arabidopsis*. The total number of ARL GTPases was way too high and 2 sets of four ARLs were clustered separately. The analysis revealed that there may be errors either in TC-Ids, repetition, duplication or the sequences might belong to a separate ARL Like or ARF related proteins as at least five tomato ARLs clustered together with *Arabidopsis* ARLs. This will need further investigation and the new release or the analysis of SGN

database may finally reveal the exact number of ARLs in tomato (discussed in the later section of SGN ITAG2.4 analysis of Solyc IDs). Similarly 7 putative Tomato SAR GTPase were identified as compared to three in *Arabidopsis*, out of which two were falling in cluster with *Arabidopsis* SAR GTPases. Thus it can be said with certainty that there are atleast 2 SAR GTPases in tomato. The other sequences needs further analysis as discussed above. A total of four RAN and 9 ROP GTPases were identified in tomato as compared to 2 RAN and 11 ROPs in *Arabidopsis* as evident from the phylogeny.

The regulators of RAB GTPases were also analysed. A total of 20 *Arabidopsis* Rab GAPs were used for the comparative analysis which resulted in identification of 23 putative Tomato Rab GAPs. Out of 23 tomato RabGAPs at least 14 were falling in cluster with *Arabidopsis* RabGAPs, which again highlighted the diversity among sequences of the RabGAPs. GAPs were also difficult to align as was observed in the comparative MSA. The accurate characterization of individual Rab GAPs is required in order to find which GAPs are involved in the regulation of the specific classes and individual groups of Rab GTPases, which is still elusive and there are very few reports for the same. The analysis of Rab GEF led to the identification of only two tomato Rab GEFs. Whereas in case of Rab GDI four *Arabidopsis* RabGDIs were used as references which led to identification of 6 putative tomato RabGDIs out of which only 3 were falling in same clade with *Arabidopsis* RabGDIs, this again needs further investigation and analysis of the tomato Genome database (SGN) which may yield further insight and give more clear results. These were the preliminary results from TIGR DFCI TGI; the analysis of future releases from SGN database may reflect the actual number of Rab GTPases, other small GTPases and regulators of Rab GTPase in tomato.

Analysis of Rab GTPases from the tomato genome database (SGN-ITAG2.4)

The results of preliminary analysis of the tomato SGN Database indicated a total 55 putative Rab GTPases (table3.18) as compared to 57 *Arabidopsis* Rab GTPases (Table3.19). They were grouped into eight functional classes with five Rab1/D, five Rab2/B, four Rab5/F, three Rab6/H, four Rab7/G, five Rab8/E, twenty five Rab11/A and four Rab18/C. The available functional annotation of SolycIds of Rab GTPases from SGN database was also compiled with their location, start and

end sequence IDs, and strands (+ve or -ve) (table 3.20.1 to 3.20.8), which confirmed their sub class specific group. This is the first report of tomato Rab GTPases from SGN database with ITAG-2.4 release. Recently SGN released new update for tomato ITAG-3.1 (3rd March 2017). The results of the analysis presented in the thesis stands true and as such with the same Solyc Ids of tomato Rab GTPases.

Other reports of classification of Rab GTPases from *Populus trichocarpa* genome sequence for the small GTP-binding gene orthologues yielded a total of 117 sequences; 31 ARF/SAR, 68 RAB, 14 ROP, and four RAN (Tuskan *et al*., 2006). In grapes Abbal *et al*., (2008) identified a total of 26 VvRab GTPases and out of these a total of 14 RabA GTPases were found along with three RabB, one RabC, one RabD, two RabE, three RabF, one RabG and one RabH. Ostlund *et al*. (2010) identified a total of 766 sequences from 11 organisms that correspond to orthologues of the 167 human proteins in the super family by using the InParanoid software (v.4.0). Wang *et al*., (2014) identified and analysed 52 Rab GTPases from channel catfish and studied their involvement in immune responses after bacterial infections. Gallegos *et al*., (2012) and Reiner and Lundquist, (2016) analysed, identified and classified the Rab family of GTPases in *C. elegans.* Surkont and Pereira-Leal (2016) reported the analysis of Rab GTPases in Archaea. Likewise Uno *et al*., (2016) identified and analysed Rab GTPase family in the brain's *corpus allatum* of *Bombyx mori.* Bright *et al*., (2017) reported the functional diversification in the Rab GTPase gene family in *Paramecium* species. Wang *et al*., (2017) analysed small GTPases in *Nilaparvata lugens* and the gene expression patterns during nymphal development. Recently Li and Guo (2017) reported the genome wide characterization of Rab GTPases in Cotton (*Gossypium* sp.). They were able to identify a total of 87, 169, 136 and 80 Rabs in the four sequenced cotton species, *G. raimondii*, *G. hirsutum* acc. TM-1, *G. barbadense* acc. 3-79 and *G. arboretum.* These reports indicate that Rab GTPases although conserved through evolution but individual groups have evolved different functions based on cell type and organism which is reflected by difference in the numbers of Rab GTPases family and sub family members. The above reports highlight the case that very few reports of the analysis of the complete set of Rab GTPases in higher plants are available till date as compared to the other organisms e.g. bacteria, insects, fish, mammals, insects and fungi.

RabA/Rab11

The detailed report of tomato Rab11a/RabA1a highlights all the Rab family specific and Rab sub family specific motifs characteristic of Rab11/A. A total of 25 Solyc RabA GTPases were found in tomato. Solyc Rab11/A was further classified into 6 sub groups from A1 to A6. The results of classification led to identification of seven RabA1, six RabA2, one RabA3, five RabA4, five RabA5 and one RabA6. The results are in agreement with the reported *Arabidopsis* RabA GTPases (Rutherford and Moore, 2002; Vernoud *et al*., 2003) where they were able to classify a total of 26 *Arabidopsis* RabA GTPases into six classes with nine RabA1, four RabA2, one RabA3, five RabA4, five RabA5 and two RabA6. In rice, there are 17 Rab-As within 52 Rab GTPase family members (Ebine and Ueda, 2009). Out of total 52 and 40 Rab GTPases from Rice and Mazie a total of 17 and 15 were grouped into RabA GTPases respectively (Zhang *et al*., 2007). Falchi *et al*., (2010) reported six RabA from peach. Recently, Li and Guo (2017) classified Rab GTPases from *Gossypium ramondii* and reported that out of 87 Rab GTPases, 34 belonged to RabA class with nine RabA1, eight RabA2, two RabA3, six RabA4, seven RabA5 and two RabA6 GTPases.

The MSA of RabA1a (fig.3.30) from different crop species including tomato, Medicago, Soybean, *Arabidopsis* confirms the high homology and characteristic motifs conserved among the species. Table no. 3.21 lists the total tomato RabA GTPases with Solyc ids, locus name, chromosome location, nucleotide length, mRNA length, Amino acid length, no. of exons, and putative automated annotations. Out of 26 RabA GTPases Solyc10g050030 was excluded because of incomplete sequence (only 80 aa length) (table7.1). The digital expression profile of Solyc RabA1a (Solyc07g055290) from various tomato tissues was obtained from SGN database, which gave a fair idea of differential expression pattern across tissues. The next step was to align the protein sequences of 25 RabA GTPases (fig.3.32) which in turn was used to generate a phylogenetic tree (fig. 3.33). The individual classes of RabA GTPases ranging from A1-A6 clearly distinguish the sub groups of RabA GTPases confirming the results of classification. Likewise the protein domains of RabA GTPases (fig.3.34) were also generated from SGN database to highlight the conserved Rab sub family specific domains. The phylogenetic tree of *Arabidopsis* and tomato RabA GTPase was generated from the MSA (fig.3.36) where in the individual classes of RabA

from Arabidopsis falls in the same cluster and clades with tomato RabA GTPases further confirming the analysis and classification.

RabA1

The tomato Rab11/A was also found to be the largest group among the Rab GTPase and among RabA sub group with seven members RabA1 had the highest members as compared to other RabA GTPases. This highlights the diversity and evolution of paralogues for carrying out a particular cellular function. So far the functional and co-localization studies of the members of RabA GTPases have only been reported in *Arabidopsis*. RabA is localized to TGN and is involved in cell plate formation and tip growth (Preuss *et al.*,2004; Chow *et al.*,2008; Szumlanski and Nielsen, 2009; Bottanelli *et al.,* 2011; Kang *et al.,* 2011; Feraru *et al.,* 2012; Asaoka *et al.,* 2013; Choi *et al.*,2013 Berson *et al.,* 2014; Kirchhelle *et al.*,2016). *RAB-A1a*, *RAB-A1b*, *RAB-A1c* and *RAB-A1i* have distinct but overlapping expression in rapidly growing tissues where active membrane trafficking is required (Kotzer *et al*., 2004). RabA1a was involved in the regulations of PIN1 and PIN2 from endosomes to PM (Koh *et al*., 2009). In the BEX5 mutant AtRabA1 was involved in the recycling of vesicles of PM (Feraru *et al*., 2012). RabA and VHA-a1 were partially co-localized to the TGN (Chow *et al*., 2008; Naramoto *et al*., 2014). At the Golgi interface Rab11 interacts with the acto-myosin for the vesicle biogenesis and trafficking (Miserey-Lenkei, *et al*., 2010). RABA1a was reported to participate in the trafficking of pectin deposition to the cell wall and fruit softening in tomato (Lunn *et al*., 2013). RABA2, RABA1c, RABA1d, and RABA1e are recruited to the cell plate during cytokinesis in *Arabidopsis* root tips (Chow *et al*., 2008, Asaoka, *et al*., 2012, Qi *et al*., 2013, Oda *et al*., 2014, Berson *et al*., 2014). The expression of *RAB-A1h* and *RAB-A1i* was pollen-specific, *RAB-A1e* was strongly expressed in roots, but other members were ubiquitously expressed. Null mutants of individual RabA genes impact the proportion of different cell wall components in stem tissue of *Arabidopsis thaliana* (Lunn *et al*., 2013). AtRabA1 was also implicated in salinity stress tolerance (Asaoka *et al.,* 2013). RabA was also reported to be involved in the subcellular trafficking of the FLAGELLIN SENSING2 receptor (Choi *et al.,* 2013). Knockout mutants of RabA had a considerable impact on the cell wall composition and saccharification efficiency in stem tissue of *Arabidopsis* (Lunn *et al.,* 2015). This report yielded an important insight into the possible

trafficking of the cell wall related enzymes which could play an important role in the cell wall remodelling during the later stages of fruit ripening which results in fruit softening, thus inhibition of the trafficking carried out by this group may somehow be related to the phenotype where in tomato fruits showed delayed pattern of softening as was observed in the present investigation. The above reports highlight the diverse function and sub cellular localization of different RabA group members in the membrane trafficking. It is still not known if the same sub-group members compensate for the loss of one of their group members. The individual regulators of these members are still elusive. It would be interesting to see if a screen can identify the individual RabA regulators as well as effectors and which proteins are individually trafficked by these members to come to a more concrete conclusion about the individual protein trafficking in the cell by RabA GTPases.

RabA2 and RabA3

RabA2 had six members and RabA3 had only one member in tomato. So far there are no reports on RabA3 GTPases apart from one, where, RabA2 and RabA3 GTPases were reported to define a trans-Golgi endosomal membrane domain in *Arabidopsis* that contributes to the cell plate formation (Chow *et al.,* 2008). RAB-A2a is also required for the root hair formation and nodulation (Blanco *et al*., 2009). RabA2 RNAi knock out impairs root hair formation and growth as well as affects pre-infection events of symbiosis in common bean (*Phaseolus vulgaris*) (Blanco *et al*., 2009). RabA2 is required for progression and maintenance of membrane integrity of infection threads during root nodule symbiosis (Dalla Via *et al.,* 2017). In case of RabA2 and A3 most of the reports are from root studies in *Arabidopsis*, it would be interesting to see what role RabA2 and RabA3 play in tomato fruit ripening and softening. As the above reports suggest their involvement in the cell plate formation which directly indicated their role in the cell division and cytokinesis, this is suggestive of their involvement during the rapid cell division phase where tomato fruit growth and expansion phase from immature green to mature green is taking place. A study of the knock out mutants of RabA3 in tomato fruits can highlight their possible role and if they are associated with any phenotype. Since RabA2 group has six members it would be important to identify the candidate before undertaking the transformation either by gene networks or co-expression analysis at the developmental stages of fruit

ripening. The role of RabA3 and RabA2 can also be studied by transient transformation in the protoplasts of tomato by tagging them with makers to observe the trafficking and a potential study can be undertaken to isolate the vesicles which are directed by RabA2 and RabA3 and to find out which proteins are being trafficked by them.

RabA4

A total of five RabA4 members were identified in tomato in the present study. RABA4b acts as an effector of phosphatidylinositol-4 kinase (PI-4K) (Preuss *et al*. 2006). RabA4B specifically interacts with phosphatidylinositol 4-kinase b1 (PI4Kb1) and PI4Kb2, and these proteins coordinate to regulate polarized expansion in root hairs (Preuss *et al*., 2006). Mutants of RabA4 were defective in endocytosis, sorting and recycling of vesicles at TGN and MVB (Choi *et al*., 2013). Interaction of AtRabA4c with PMR4 effector resulted in complete penetration resistance to powdery mildew fungus (Ellinger *et al.,* 2014). Recruitment of PLANT U-BOX13 and the PI4Kβ1/β2 phosphatidylinositol-4 kinases by AtRabA4B plays important roles during salicylic acid-mediated plant defense signalling (Antignani *et al.,* 2015). Thus RabA4 subgroup has been shown to be involved in the intra-cellular trafficking involved in the polarization of cell growth as well as in biotic and abiotic stress tolerance. It would be interesting to see if RabA4 group members also play an important role in the disease resistance to the gray mould fungus in tomato, which need further investigation. A patho-physiological study of the wild type and the knock out mutants of RabA4 members can be carried out and checking the gene expression profiles of these members after inoculating the fruits with the spores of gray mould. Imaging of the host and pathogen interaction in the normal and infected tomatoes can shed light if the knock out of RabA4 members is involved in the disease resistance.

RabA5

A total of five RabA5 members were identified in tomato. AtRabA5c (ARA4) showed a specific expression in the exocarp of ripe grape berries, in all seven red and white berry varieties (Abbal *et al*., 2008). VvRabA5e was shown to be developmentally regulated in grapes (Conéjéro *et al.,* 2010). *Arabidopsis* 'chloroplast localized Rab GTPase' protein CPRabA5e is involved in stress, development, thylakoid biogenesis and vesicle transport (Karim *et al.,* 2014). Recently RabA5c was reported to be involved in the trafficking to the cell

boundaries/edges and in the regulation and maintenance of the plant cell shape (Rahni *et al*., 2016, Kirchhelle *et al*., 2016). It would be interesting to see if the knockout of RabA5 member does have an impact on the fruit shape or if they are involved in the deformed and rumpled leaves found in the tomato RabA1a antisense lines. Expression profiles from the exocarp of tomato fruits at different developmental stages of ripening of normal and knock out mutants of RabA5 should be generated and associated phenotypes should be observed with respect to the changes in cell wall composition of the exocarp and shape of tomato fruit. Imaging of fluorescent tagged RabA5 members and the vesicles trafficked to the cell boundaries in exocarp can shed more light in the trafficking of cell wall components by RabA5 members.

RabB, C, D, E, F, G and RabH

The MSA of Rab1, 2, 5, 6, 7, 8 and Rab18 was analysed and individual groups were clustered together (highlighted in fig.3.37) and a phylogenetic tree for the same Rab GTPases revealed that the sequences of each individual groups fall together in the same clade (fig.3.38). Similarly MSA (fig.3.40) and the phylogenetic tree (fig. 3.39) of *Arabidopsis* Rab GTPases as references were used to compare the tomato Rab GTPases. Each individual tomato Rab Sequences fall within the same clade of *Arabidopsis* Rab GTPases confirming the results of classification. To validate the results all tomato Rab GTPases were included in analysis to generate MSA and tree (fig. 3.40.1 and 3.40.2) and likewise all tomato and *Arabidopsis* Rab GTPases were again analyzed to include all eight classes of Rab GTPases (fig3.40.3 and 3.40.4). The analysis and classification stands true for all Rab GTPases classified even with the latest release of SGN-ITAG3.1 (March, 2017).

Rab2/RabB

The initial analysis of Rab2/Rab B GTPases from the SGN database (ITAG2.4) revealed a total of five Rab2/B GTPases in tomato (table 3.22). In *Arabidopsis*, there are three Rab2/B members (Rutherford and Moore, 2002; Vernoud *et al*., 2003). Zhang *et al*., (2007) reported 4 RabB from Rice and 3 from Maize whereas, Falchi *et al*., (2010) found at least 2 RabB GTPases in Peach. Li and Guo (2017) found 6 RabB GTPases in *G. ramondii.* A Rab-B (NtRab2) in tobacco pollen was reported to localize to Golgi bodies and regulates trafficking between

the ER and Golgi (Cheung *et al*., 2002). RAB2A controls MT1-MMP endocytic and E-cadherin polarized Golgi trafficking in breast cancer (Kajiho *et al.*, 2016). Rab2 and RabS co-localize with the Golgi and contractile vacuole system and are involved in the osmoregulation in *Dictyostelium discoideum* (Maringer *et al.*, 2016). Recently Rab2 was also reported to promote autophagic and endocytic lysosomal degradation (Lőrincz *et al.*, 2017). It would be interesting to see which proteins are being trafficked by the vesicles associated with the Rab2/B members by utilizing the imaging techniques either by generating the antisense transgenic lines or by studying the protoplast of tomato fruits with fluorescent markers labelled to Rab2/B. The screening of the individual members and a gene co-expression network with the cell wall hydrolases followed by a mass spectrometric analysis of vesicles harbouring the Rab2/B in normal and knock out mutants of Rab2/B in tomato at different developmental stages can shed light on the possible role played by them in intra-cellular trafficking. The above reports also suggest their role in osmoregulation and autophagy, this can well be studied by exposing the tomato plants in one experiment and the protoplasts in other screens to salt stress a different concentrations to see if there is any difference between the wild type and transgenic Rab2/B lines.

Rab18/RabC

A total of five Rab18/RabC were found in tomato and one (Solyc07g052410) was excluded from the present study. The annotation in SGN still mentioned it as Rab18 but further analysis and BLAST results from other relevant database at NCBI revealed 'Solyc07g052410' to be a septum promoting GTPase (SPG) (table7.1). Although it has the domains of GTPases and GTP binding sites but it does not qualify as Rab18 GTPases hence it was excluded from the study. Finally 4 tomato RabC GTPases were compared to three *Arabidopsis* RabC (Rutherford and Moore, 2002; Vernoud *et al*., 2003). The most similar protein of RabC in mammals is Rab18, which is implicated in the endocytic transport (Segev, 2001; Zerial and McBride, 2001). Zhang *et al*., (2007) reported 3 RabC from Rice and 4 from Maize whereas, Falichi *et al*., (2010) found at least 2 RabC GTPases in Peach. Li and Guo (2017) found 8 RabC GTPases in *G. ramondii.* Rab18 was localized to the lipid droplets and ER derived membranes (Ozeki *et al*., 2005). Rab18 and its GEF are required for normal ER structure (Gerondopoulos *et al*.,

2014). MiR-455-5p down-regulates Rab18 and acts as a tumor suppressor in gastric cancer (Liu *et al*., 2016). Rab18 facilitates dengue virus infection by targetting fatty acid synthase to sites of viral replication (Tang *et al*., 2014). Feldmann *et al*., (2017) reported the involvement of Rab18 in macro-autophagy and proteostasis. Rab18 was reported to be involved in the salinity stress response in rice (Jha *et al*., 2014) and in case of watermelon (*Citrullus lanatus*), it was highly expressed in root, stem and leaf, moderately expressed in flower but weakly expressed in the fruit (Xinli and Lei, 2015) whereas in case of transgenic wheat over-expressing *TaRab18* showed a strong hypersensitive response at the early infection stage of wheat stripe rust (Jiang *et al*., 2017). These reports indicate the possible involvement of Rab18/C in abiotic stress (osmoregulation) and biotic stress (autophagy and hypersensitive response). Most of the reports on Rab18/C are from the studies in mammalian system, it raises an important point to study the role of Rab18/C in the salinity tolerance in tomato. This can be investigated by comparing expression profile of wild type and transgenic Rab18/C members in tomato by exposing them to different salt stresses/regimes to study their effects in abiotic stress response. In case of present study on RabA1a antisense transgenic lines the enhanced shelf life associated with prolonged water retention and maintenance of fruit firmness also provides a platform for future study to find the interaction or the co-expression networks of RabA and RabC in order to see if there is any correlation and a possible role in the osmoregulation by RabC in antisense transgenic tomato fruits. In-order to study the role played by them in biotic stress response the fruits of wild type and transgenic plants should be inoculated with the spore of gray mould fungi to check if they are capable of inciting a hypersensitive response in the tissues or by using the protoplasts of wild type and transgenic Rab18 members inoculated with spores to check the germination and invasion of the fungal hyphae by microscopy and live cell imaging.

Rab1/RabD: There were five Rab1/D GTPases in tomato as compared to four RabD in *Arabidopsis*, with a single-member in RabD1 subclass and three in RabD2 (Vernoud *et al*., 2003). Zhang *et al*., (2007) reported 7 RabD from rice and 8 from maize whereas, Falchi *et al*., (2010) found 2 RabD GTPases in Peach. Li and Guo (2017) found 7 RabD GTPases in *G. ramondii*. Rab-D is related to Ypt1

and Rab1 in yeast and mammals, respectively (Rutherford and Moore, 2002; Vernoud *et al*., 2003). Yeast Ypt1 and Rab1 in animals act in ER to Golgi transport and early Golgi transport by regulating COPII vesicles tethering and fusing to *cis*-Golgi (Allan *et al*., 2000; Cooper *et al*., 2006). In-vivo imaging analysis indicates that RabD proteins localize to the Golgi and the TGN (Geldner *et al*., 2009; Pinheiro *et al*., 2009). Expression of the dominant negative form of RabD1 and RabD2 revealed the function of RabD proteins in ER to Golgi anterograde transport (Batoko *et al*., 2000; Pinheiro *et al*., 2009; Saint-Jore *et al*., 2002). AtRabD2b and D2c play important roles in pollen development, germination, pollen tube elongation and are highly expressed in pollen and the mutants of *AtrabD2b/2c* produced deformed pollen with swollen and branched pollen tube tips (Peng *et al*., 2011). Involvement of RabD GTPase in wheat and its role in gluten quality was recently reported by Tyler *et al*., (2015). In *Plasmodium falciparum* Rab1A was localized to Rhoptries in Schizonts (Morse *et al*., 2016). Recently Lukman *et al*., (2017) used clustering methods to identify Rab1 binding sites. It would be interesting to see if the same clustering method can be employed in tomato to identify the Rab1/D binding sites. Rab1/D have been analysed in few plants as compared to animals as discussed above, but haven't been characterized for their possible function and role in trafficking in plants apart from *Arabidopsis*, cotton and wheat. This presents a new topic of research to investigate the possible role played by Rab1/D in tomato fruit development. The post fertilization events need to be studied by conducting a morpho-physiological assessment and imaging to see if there is any difference in the pollen development, maturation, pollen tube elongation after pollination. It would be interesting to see if the pollens from the transgenic plants affect the number of fruits on the truss as well.

Rab8/RabE

There were five Rab8/E GTPases in tomato as compared to five RabE in *Arabidopsis* (Vernoud *et al*., 2003). Zhang *et al*., (2007) reported 6 RabE from rice and 4 from maize whereas, Falchi *et al*., (2010) found atleast 1 RabE GTPases in peach. Li and Guo (2017) found 8 RabE GTPases in *G. ramondii.* Rab-E is homologous to Rab8 and Rab10 in mammals and to Sec4 in *S.cerevisiae* (Rutherford and Moore, 2002; Vernoud *et al*., 2003). However, despite the

sequence similarity, the five *Arabidopsis* RabE proteins may not have conserved function as Rab8 and Rab10. A RabE GTPase mutant acts downstream of the RabD and is involved in the traffic to the PM in tobacco leaf epidermis (Zheng *et al*., 2005). AtRabE GTPases exhibit a novel interaction with a PM phosphatidylinositol-4-phosphate 5-kinase (Camacho, *et al*., 2009; Speth *et al*., 2009; Wall *et al*., 2017). Rab8a regulates the exocyst mediated kiss and run discharge of the contractile vacuole in *Dictyostelium* (Essid *et al*., 2012). Ahn *et al*., (2013) Characterized NbRabE1 from *Nicotiana benthamiana* and Gao *et al*., (2016) characterized, isolated and studied the expression pattern of At RabE1d13-185. Recently Vidal-Quadras *et al*., (2017) reported the role of Rab8 in the cell polarization. So far no reports are available which can highlight the possible role of Rab8/E in tomato fruit ripening. It would also be interesting to see if Rab8/E in tomato also interacts with PIP kinases by generating a co-expression network at different fruit developmental stages.

Rab5/RabF

A total of six Rab5/RabF were found in tomato and two (Solyc05g045660 and Solyc12g005840) were excluded from the present study. The annotation in SGN still mentioned them as Rab5 but further analysis and BLAST results from other relevant database at NCBI revealed Solyc05g045660 and Solyc12g005840 both to be septum promoting GTPase (SPG) and not Rab5 GTPase (table7.1). The error might be due to the automated annotations as discussed earlier. Although it has the domains of GTPases and GTP binding sites but it does not qualify as Rab5/F GTPases hence they were excluded from the study. Thus, tomato has four Rab5/F GTPases as compared to three in *Arabidopsis*, which are classified into RHA1 (RABF2a), ARA7 (RABF2b), and ARA6 (RABF1) (Ueda *et al.*, 2001, 2004, Saito and Ueda 2009). Zhang *et al*., (2007) reported 7 RabF from rice and 3 from maize whereas, Li and Guo (2017) found 9 RabF GTPases in *G. ramondii.* The difference in the number of Rab genes are mainly caused by gene duplications, leading to the presence of paralogs (e.g. Rab5a, Rab5b and Rab5c).

Extensive studies of Rab5 in plants demonstrate that Rab5 mainly localizes to PVCs and functions in vacuolar trafficking (Goh *et al*., 2007; Ebine *et al*., 2011; Bottanelli *et al*., 2012; Inoue *et al*., 2013; Jia *et al*., 2013).

Table7.1 List of Solyc IDs of GTPases Excluded from the analysis based on NCBI results and Incorrect Annotation in SGN Database

Solyc ID	Annotation in SGN	Location(s)	DNA	mRNA	AA	Strand	Remarks
Solyc05g045660 Solyc05g045660.2.1 is not a Rab5	Solyc05g045660 Rab5 acc sgn Ras-related protein Rab-5C (AHRD V1 ***- B5X4R4_SALSA); contains Interpro domain(s) IPR003579 Ras small GTPase, Rab type SGN-U564409 Tomato 200607 -- build 2 -- 3 members	SL2.50ch05:57401079..57403786	2708	963	213	+	SPGTPase
Solyc12g005840 Solyc12g005840.1.1 is not a Rab5	Solyc12g005840 Rab 5 acc sgn Ras-related protein Rab-5C (AHRD V1 *-*- B5X4R4_SALSA); contains Interpro domain(s) IPR003579 Ras small GTPase, Rab type	SL2.50ch12:474797..477406	2610	903	301	-	SPGTPase
Solyc10g050030 Solyc10g050030.1.1 is incomplete Rab11	Solyc10g050030 Rab11 acc sgn Ras-related protein Rab-25 (AHRD V1 ***- RAB25_BOVIN); contains Interpro domain(s) IPR015595 Rab11-related	SL2.50ch10:47634900..47636259	1360	240	80	+	Incomplete Sequence
Solyc07g052410 Solyc07g052410.2.1 is not a Rab18	Solyc07g052410 Rab 18 acc sgn Ras-related protein Rab-18 (AHRD V1 ***- B6TGJ7_MAIZE); contains Interpro domain(s) IPR003579 Ras small GTPase, Rab type	SL2.50ch07:60927071..60929129	2059	988	258	+	SPGTPase

SPG: Septum Promoting GTPases (Remarks-as per BLAST results indicate from the NCBI Database) March, 2017

So far, *ARA6* homologues have been identified in the genomes of angiosperms, the lycophyte *Selaginella moellendorffii* and the bryophyte *Physcomitrella patens.* Rab5 was highly expressed in *Mesembryanthemum crystallinum* during early salt stress (Bolte *et al*., 2005). These reports are indicative of the possible role played by Rab5/F in salt stress in plants as they were also reported to be localized to PVC and tonoplast and the trafficking towards them. These reports build up a case right from the lower plants to the higher plants specifying their conserved function and expression during the salt stress. It would be interesting to see if over expression or knock out of the Rab5/F has a significant impact on the survival of tomato seedling during the salt stress.

Only conventional Rab5 members were found in green algae and ARA6 was therefore reported to be specific to land plants (Ebine and Ueda, 2009). Marion *et al*., (2013) reported the biochemical and molecular characaterization of CaRABF1 (CaARA6) a member of the green algae *Chara australis* which was localized at the PM, TGN and MVEs. Hoepflinger *et al.*, (2015) analysed the CaARA7 (RAB5) GTPase in Characean Algae. AtARA6 has been shown to localize at multivesicular endosomes (Haas *et al.*, 2007, Wang *et al.*, 2009). Recent studies indicate that ARA6 also localizes to the PM, where it plays a regulatory role in the formation of a SNARE complex containing VAMP727 and SYP121 (Ebine *et al*., 2011). This should be further investigated in tomato in case if the gene co-expression network can find a link between Rab5/F and SNAREs in tomato.

A mutated form of ARA6 has also been shown to perturb vacuolar trafficking of soluble cargos in tobacco cells (Bottanelli *et al.*, 2011). The *Arabidopsis* double mutants of Rab5 (RHA1 (RABF2a), ARA7 (RABF2b)) or mutants lacking functional Rab5 GEF VPS9a were found to be non-viable (Goh *et al*., 2007; Ebine *et al*., 2011). GLUP4/Rab5a and its GLUP6/GEF are involved in the intracellular transport of the pro-glutelin from the Golgi to the PSV (Fukuda *et al.*, 2011, 2013). SAND/MONENSIN SENSITIVITY1 and CALCIUM CAFFEINE ZINC SENSITIVITY1 (CCZ1) act as effector of RAB5 and GEF for Rab7 (Cui *et al*., 2014; Ebine *et al*., 2014; Singh *et al*., 2014). The above reports set the stage for an investigation of tomato Rab5/F where the regulators of Rab5/F in tomato still need to be identified and characterized in order to find their regulation and interaction at different stages of tomato ripening. It would also interesting to see if

the GEFs in tomato can also hop and act as effector for the other Rab as from Rab5/F to Rab7/G.

Liu *et al*., (2014) reported the molecular characterization and expression analysis of MiRab5 in *Mangifera indica*. AtRAB5/ARA6 is required for starch and sugar homeostasis through the function of Qua-Quine Stach gene (QQS), and was also involved in the proliferation of *Pseudomonas syringae* pv. tomato DC3000 which was repressed in AtRabF1 knockout mutant (Tsutsui *et al*., 2015). AtRab5 and its interacting partner ENDOSOMAL RAB EFFECTOR WITH PX-DOMAIN (EREX) regulates trafficking to protein storage vacuoles (Sakurai *et al*., 2016). ARA6/RABF1 and ARA7/RABF2b were localized to extra-haustorial membrane (EHM) formed by the *Arabidopsis* powdery mildew fungus *Golovinomyces orontii* (Inada *et al*., 2016). Amir *et al*., (2016) isolated and characterized *TcRab5a* from the medicinal plant 'heart-leaved moonseed' (*Tinospora cordifolia*) belonging to the family menispermaceae. Recently the role of AtRabF1 was elucidated in salt stress and dark-induced senescence (DIS) in *Arabidopsis* (Yin *et al*., 2017). The above reports indicate the possible role of Rab5/F in the biotic and abiotic stress as in case of sugar homeostasis and response to the bacterial and fungal infection which needs to be investigated in tomato fruits.

RabG/Rab7

Four Rab7/G GTPase were found in tomato as compared to eight members in *Arabidopsis* (Rutherford and Moore, 2002; Vernoud *et al*., 2003). Zhang *et al*., (2007) reported 5 RabG from rice and 3 from maize whereas, Falchi *et al*., (2010) found one RabG in Peach and Li and Guo (2017) found 9 RabG GTPases in *G. ramondii.* The presence of Rab7/G GTPases in different lower or higher plants indicate the essential role played by them during the course of evolution as well as the difference in the number is indicative of the variation which may have arisen because of speciation, duplication, drift, mutation or selection. This may well be related to the genome size and moreover to the specific functional related needs of the cells, different cell types and tissues during the evolutionary process for the survival and maintenance of the cell and organism as a whole. The size of genomes with respect to the ploidy of the organism (diploid or polyploidy e.g. tomato (2x), potato (4x), wheat (6x)) which can have multiple copies of the same

gene on different chromosomes sets can well have a profound effect on their expression. This might be the reason the numbers vary from plants to plants. In plant cells, Rab7 proteins are thought to be localized to the tonoplast, which mediates the final step of vacuolar trafficking (Nahm *et al*., 2003; Mazel *et al*., 2004; Limpens *et al*., 2009; Rho *et al*., 2009; Heo *et al*., 2010; Kwon *et al*., 2010). Cui *et al* (2014) reported that AtRABG3f localizes to PVCs and tonoplast. The transgenic plants over expressing AtRab7 and PgRab7 showed increased tolerance to salt and osmotic stresses (Mazel *et al*., 2004; Agarwal *et al*., 2008). Recently Sui *et al*., (2017) reported that Rab7 was involved in salinity stress resistance in peanut. The above reports from different plants indicate that Rab7/G is largely involved in the biotic and abiotic stress response. It raises an interesting question in case if Rab7/G is involved in the homeostasis and water retention in tomato fruits in normal wild type fruits and if it is also involved in the altered behaviour and prolonged water retention in the fruits of Rab11a antisense transgenic fruits where the fruits retained firmness for a longer duration and showed pattern of delayed fruit softening.

The transgenic RabG3bOX (over expression) and RabG3bCA (constitutive active) plants displayed unrestricted hypersensitive programmed cell death (PCD) against a fungal toxin Fumonisin-B1 and *Alternaria brassicicola* (Oh *et al*., 2005; Kwon *et al*., 2009). OsRab7B3 enhances leaf senescence in transgenic rice (Pitakrattananukool *et al*., 2012). AtRabG3b was shown to positively regulate autophagy and immunity associated hypersensitive cell death (Kwon *et al*., 2013). NbRABG3f was shown to be involved in Bamboo mosaic virus infection in *Nicotiana benthamiana* (Huang *et al*., 2016). A Rab7 effector PLEKHM1 was reported to bind ARL8b to promote cargo traffic to the lysosome (Marwaha *et al*., 2017). The involvement of Rab7/G in HR and PCD highlights the important role played during and post infection stages in plant which needs further investigation and characterization in tomato fruits affected by gray mould.

Rab6/RabH

Three Rab6/H GTPases were found in tomato as compared to five members in *Arabidopsis* (Rutherford and Moore, 2002; Vernoud *et al*., 2003). Zhang *et al*., (2007) reported 3 RabH from rice and 1 from maize, whereas Li and Guo (2017) found 6 RabH GTPases in *G. ramondii.* Homologues of the five AtRabH GTPases

subfamily are Rab6 in animals and Ypt6 in yeast (Rutherford and Moore, 2002; Vernoud *et al*., 2003). Rab6 is involved in the trafficking from Golgi to ER (Storrie *et al.*, 2000). AtRAB-H1b and AtRAB-H1c target reporter proteins to Golgi when expressed in tobacco and *Arabidopsis* (Johansen *et al*., 2009). Rab6 regulates KIF1C motor domain for tethering of vesicles to Golgi (Lee *et al*., 2015). Recently Yang and Zhang, (2016) reported the involvement of OsRab6a in iron homeostasis in rice. The exact role played by Rab6/H GTPases in plants is still elusive. According to the last report of its involvement in iron homeostasis it raises questions about the need for further investigation and characterization. A possible gene and co-expression network analysis may be able to find the putative interacting partners and co-expressed genes to chart the pathway of its involvement in trafficking.

in silico analysis of other small GTPases involved in intracellular vesicle/membrane trafficking in tomato

Along with the analysis of Rab GTPases, other small GTPase such as ARF, ARL, RAN, ROP and SAR which are also involved in the various processes of trafficking were also identified from tomato. The *in silico* analysis revealed that they are also present in tomato and might have distinct physiological functions.

RAN GTPase

Analysis of RAN GTPase in tomato (ITAG2.4) led to the identification of four RAN GTPases as compared to four *Arabidopsis* RAN GTPase (fig.3.41 and table 3.23). The Ras-like nuclear (Ran) GTPase is involved in the nucleocytoplasmic transport of both RNA and proteins (Weis, 2003). Ran-GTP in the nucleus facilitates the directionality of nuclear import and export (Li *et al*., 2003). The transgenic plants over expressing OsRAN2 in rice and *Arabidopsis* were hypersensitive to salinity and osmotic stress (Zang *et al*., 2010). OsRAN1 was reported to be involved in plant cold resistance and development in rice (Xu and Cai, 2014) similarly transgenic tobacco plants over expressing *LlaRan* from *Lepidium latifolium* L. were hypersensitive to cold stress (Sinha *et al*., 2014). Li *et al*., (2016) reported that *PdRanBP* plays important role in the vascular tissue development in poplar. AtRAN1 also regulates vegetative growth and stress tolerance in *Arabidopsis* (Xu *et al*., 2016). Recently Yan *et al*., (2016) reported that *vvran1* was involved in the stress responses in the Straw Mushroom

Volvariella volvacea. Most of the reports on Ran GTPases establish its role in stress response whether it is salinity or cold. Ran GTPases need further investigation in case of tomato during the exposure of different salt or temperature regimes.

ROP GTPase

The Screening of SGN database led to identification of 10 ROP GTPases in tomato (table 3.24) as compared to 11 ROP GTPases in *Arabidopsis* (fig.3.43, fig.3.44 and fig. 3.45). Several genes encoding ROP/RAC proteins have been isolated from a number of plant species such as barley (Schultheiss *et al*., 2003), rapeseed (Chan and Pauls, 2007), cotton (Delmer *et al*., 1995; Kim and Triplett, 2004), maize (Hassanain *et al*., 2000), tobacco (Morel *et al*., 2004), and rice (Christensen *et al*., 2003). Seven ROPs in rice and 9 ROPs in maize have been reported (Li *et al.*, 1998; Winge *et al.*, 2000; Christensen *et al.*, 2003). Seven ROP GTPases from Grapes (VvRops) were classified into VvROP1, 7, 8, 9, 11, 12, 13. VvRop9 displayed high berry specificity and was responsive to ABA (Abbal *et al*., 2007, 2010; Conéjéro *et al.,* 2010). Liu *et al*., (2010) identified 7 ROPs from *Medicago trunculata.* Recently Ma *et al*., (2017) reported 6 ROPs from wheat. Plants possess a single subfamily of Rho GTPases, termed ROPs (Rho related GTPases from plants). Phylogenetic analysis suggests that ROPs evolved prior to the divergence of other three subfamilies of Rho GTPases from animals and fungi (Zheng and Yang, 2000; Vernoud *et al*., 2003). At the primary amino acid sequence level, ROPs are most similar to RACs and thus have also been referred to as RACs as in case of *Arabidopsis* (Yang, 2002; Christensen *et al*., 2003; Vernoud *et al*., 2003). ROP/RAC GTPases that control polarized cell growth have been implicated in directing exocytic vesicles via the cytoskeleton to sites of fast membrane expansion during interaction of fungal pathogens with barley (Opalski *et al*., 2005; Pathuri *et al*., 2008), *Arabidopsis* (Poraty-Gavra *et al*., 2013), and rice (Chen *et al*., 2010a), or mycorrhizal and oomycete root colonization in barley (Kiirika *et al*., 2012). Within the plant kingdom, ROPs share over 70% amino acid similarity with each other and are divided into four phylogenetic groups I–IV (Zheng and Yang, 2000; Yang, 2002). Various Rops have been shown in establishment of polarity, regulation of the actin cytoskeleton, pathogen defense responses, secondary cell wall formation, meristem signalling,

vacuole development and auxin signalling (Huang *et al*., 2014; Ren and Deshu, 2015; Schepetilnikov *et al*., 2017). AtRop2 (AtRac4), AtRop4 (AtRac5), and AtRop6 (AtRac3) GTPases are involved in root hair growth (Molendijk *et al*., 2001; Jones *et al*., 2002), and AtRop10 (AtRac8) in ABA signalling (Zheng *et al*., 2002). ROP6 and other ROPs have been suggested to localize to lipid rafts (Sorek *et al*., 2007). AtPRK2 promotes ROP1activation via RopGEFs in the control of polarized pollen tube growth (Chang *et al*., 2012). ROP6 was involved in nodule formation in *Lotus japonicus* (Ke *et al*., 2012; Wang *et al*., 2015). AtROP6 functions in developmental and pathogen response pathways. (Poraty-Gavra *et al*., 2013). SPIKE1 activates ROP GTPase to modulate petal growth and shape (Ren *et al*., 2016). MAP18 was shown to affects ROP2 activity during root hair growth (Kang *et al*., 2017). AtROP2-RIC1 pathway fine tunes microtubule reorganization for salt tolerance in *Arabidopsis* (Li *et al*., 2017). This is the first report of analysis of ROP GTPases in tomato. ROPs have been involved in the regulation of hormones as well e.g. Auxin and ABA, which highlights the need to investigate their role in the morpho-physiological parameters in tomato growth and development. The expression profiling of ROP GTPases at different developmental stages of fruit ripening and disease development can shed more light on their diverse function.

ARF

A total of 14 tomato ARF GTPases (table 3.25) were screened initially from the SGN database. Two sequences Solyc09g031870 and Solyc10g049260 both were excluded from the present study because of incomplete sequences of 50 and 60 aa respectively. Thus 12 sequences of ARF GTPases were found in tomato as compared to 12 ARFs in Arabidospsis (fig.3.46, fig3.47, fig.3.48). Like Rab GTPases the ADP Ribosylation factor (ARF) family proteins are involved in regulation of vesicular transport. The N-terminal region of ARF is myristoylated (Pasqualato *et al*., 2002, Burd *et al*., 2004; Kerr and Kahn, 2015) .The ARF GTPase superfamily also includes the ADP ribosylation factor ARF, ARL (ARF-like), ARP (ARF-related proteins) and SAR (Secretion-associated and Ras-related) proteins. There are currently 6 known mammalian ARF proteins, which are divided into three classes of ARFs: class-1: ARF1, ARF2, ARF3; class-2: ARF4, ARF5 and class-3: ARF6. GNOM is an ARF GEF which was reported to

be involved in the trafficking of PIN proteins (Teh and Moore, 2007, Naramoto, 2014).

AtARF1 plays a critical role in intracellular trafficking and maintenance of ER morphology in *Arabidopsis* (Lee *et al*., 2002). ARF1 is involved in traffic between the ER and the Golgi in tobacco and *Arabidopsis* and cell polarity (Takeuchi *et al*., 2002; Xu and Scheres, 2005; Cohen *et al*., 2010; Robinson *et al*., 2011; Donaldson *et al*., 2016). ARF1p also controls the sequence-specific vacuolar sorting route to the lytic vacuole (Pimpl *et al*., 2003).The ARFA1b/1c plays important role in callose deposition and ROR2 syntaxin dependent pre-invasive defense in barley (Böhlenius *et al*., 2010). ARF1 and SAR1 are expressed during development and the de-etiolation process (Keskin *et al*., 2012; Keskin, 2013). Marais *et al*., (2015) reported that ARF1 interacts with the Memb1 Qb-SNARES at Golgi in *Arabidopsis*. This is the first report of analysis of ARF GTPases in tomato which needs to be followed by experimental characterization to see what role they play in the intra-cellular membrane trafficking in tomato fruits. A co-expression network of the COP-I, COP-II, Rab GTPases and SNAREs involved in the trafficking should be generated to see if there is any particular ARF GTPase which plays a vital role in fruit development or disease resistance.

SAR

Four SAR GTPases were identified in tomato (table 3.26) as compared to three in *Arabidopsis* (fig.3.49, 3.50, 3.51). The SAR1 protein was first reported in budding yeast and was involved in trafficking between ER and Golgi. SAR1 is slightly related to ARFs and ARF binding proteins. SAR1 is generally included in the RAS 'superfamily' of small GTP-binding proteins, but it is only slightly related to other RAS proteins. It also differs from RAS proteins in that it lacks cysteine residues at the C-terminus and is therefore not subject to prenylation. Golgi membrane dynamics was affected in the dominant-negative mutant of NtSar1 in tobacco (Osterrieder *et al*., 2010). SAR1 regulates the assembly of COPII and is involved in trafficking of proglutelin and α-globulin from the ER to the Golgi (Tian *et al*., 2013). Sar/Arf GTPases have been shown to play important role in vesicle trafficking in yeast and plants (Yorimitsu *et al*., 2014). SAR1 was reported as novel regulator of ER-Mitochondrial contact sites (Ackema *et al*., 2016; Hanna *et al*., 2016). Recently Yavuz *et al*., (2017) reported the role for Sar1 and ARF1 in

Golgi biogenesis in *Trypanosoma brucei*. The exact functional role played SARs needs to be investigated in plants.

ARL GTPase

Seven ARL GTPases were found in tomato initially but 'Solyc00g020990' was not included because of sequence length (388 aa) and since 'Solyc00' indicates that until ITAG2.4 it was not mapped and assigned to any chromosome, but it had the 'IPR006689' domain specific to ARL GTPases. Future release and upgraded version of the database may clarify the true identity of the sequence. The phylogenetic analysis grouped ARLs into three groups with one ARLB, one ARLC and four ARLA in tomato. The ARL (ARF-like) group does not show a consistent phylogeny, as was observed while analysing the ARLs in TIGR and SGN database confirming previous observations as reported by Kahn *et al*. (2006). A total of 16 mammalian ARLs have been identified based on sequence analysis (Gillingham & Munro, 2007). ARLs have 40-60% sequence identity with each other and with the ARF family (Ingley *et al*., 1999). However, despite the conservation of the N-terminal glycine in ARL1, 2, 3, they are not substrates of N-myristoyltransferases. ARL2 and ARL3 regulate the transport of membrane associate proteins between membrane compartments (Ismail *et al*. 2011, 2012). ARL1 localises to the TGN and its effectors regulate both anterograde and retrograde trafficking pathways (Lu *et al*., 2004; Lock *et al*., 2005; Munro, 2005; Tai *et al*., 2005; Derby and Gleeson, 2007). Phylogenetic relationships indicate that ARL5 is closely related to ARL1 and ARL6 and is involved in trafficking in cilia and flagella and ARL6 was localised to the centrosome in ciliated cell lines (Li *et al*., 2004; Wiens *et al*., 2010). ARL1 and ARF RP-1 are localized to TGN where they regulate the tethering of endosome derived transport vesicles. Other ARL proteins were localized to the cytosol, nucleus, cytoskeleton, mitochondria and lysosome with diverse roles (Burd *et al*., 2004; Hofmann and Munro, 2006). Majority of reports on ARLs are from the mammalian system, which puts an impetus to investigate the role played by them in plants and to what extent they have overlapping of distinct function and expression as compared to the ARF GTPases.

Regulators of Rab GTPase

Rab GEF

In tomato only one RabGEF (Solyc01g096990) was found as compared to two in *Arabidopsis*. RabGEFs play important role in the activation of RabGTP and their targetting to the membrane (Blümer, 2013; Jackson, 2014). OsRhoGDI2 was expressed during development and abiotic stresses in rice (Huang *et al*., 2016). AtRhoGDIs plays critical role in cellular homeostasis of pollen tubes (Feng *et al*., 2016). Recently Koch *et al*., (2016) developed a pull-down assay for the identification of unknown GEFs for small GTPases. Its worth investigating if the same assay can be applied in tomato to identify GEFs for different GTPases. Rab geranylgeranyl transferase (RabGGTase) does not recognize its protein substrates (Rab proteins) directly but requires the adaptor Rab escort protein (REP). Rab prenylation requires the formation of a ternary catalytic Rab:REP:RabGGTase complex (Pylypenko , 2003, Rak *et al*., 2004; Wu, *et al*., 2009). RabGGTase mutants RabGTB1 beta subunit is expressed constitutively and has shoot growth and gravitropic defects (Hala *et al*., 2010). Recently Shi *et al*., (2016) reported the redundancy and broad substrate specificity of AtRABGGTase. The role RabGGTases play in tomato if they are over-expressed or knocked out and if there are any phenotypes associated with the same is worth investigating

RabGDI

In tomato four RabGDI and five ROP GDI's were identified in the initial analysis (table 3.27). The phyletic analysis of tomato and *Arabidopsis* RAB GDI (fig3.53) led to the identification of one SolycRabGDI1a, one SolycRabGDI2a and two SolycRabGDI1 and SolycRabGDI 2 respectively. It is interesting to note that the number of GDIs in plants is usually low as compared to the GAPs, which needs further investigation to find out if there is any effect on the turnover of the Rab GTPases if GDIs are over expressed or knocked out.

Rab GAP

In tomato initially 24 Rab GAP were found but 2 were excluded from the analysis. Solyc03g058500 (77aa) and Solyc00g117650 (82aa) were not included because of incomplete sequence and the latter being 'Solyc00' and is not yet mapped and assigned to any chromosomes in tomato. Thus screening led to the

identification of 22 Solyc RabGAP, 9 ROP GAP, 6 ARF GAP, 13 ARF GAP with ANKr PH domain and two RAN GAPs. This is the first report of GAPs of small GTPases in tomato (table3.28). The phylogeny of 22 tomato and 22 *Arabidopsis* RabGAP (fig.3.56) classified them into six different classes with five GYPA, six GYPB1, four GYPB2, four GYPC, one GYPD and two GAPTBC in tomato. RabGAPs plays important role in regulation of RAB GTPase cycle and in cellular logistics (Barr and Lambright, 2010; Kahn, 2011; Mishra and Lambright, 2016). The peculiar RabGAP-TBC where TBC stands for TBC (Tre-2/Bub2/Cdc16) domain was originally identified as a conserved domain among the *tre-2* oncogene product and the yeast cell cycle regulators *Bub2* and *Cdc16*, and it is now widely recognized as a conserved protein motif that consists of approximately 200 amino acids in all eukaryotes. Since the TBC domain of yeast GYPS [GAP (GTPase-activating protein) for Ypt proteins] has been shown to function as a GAP domain for small GTPase Ypt/Rab, TBC domain-containing proteins (TBC proteins) in other species are also expected to function as a certain RabGAP (Barr and Lambright, 2010; Fukuda, 2011; Gabernet-Castello, 2013). Structurally distinct bacterial TBC-like GAPs link ARF GTPase to Rab1 inactivation to counteract host defenses (Dong *et al*., 2012). TBC1D15 Family from *Chiloscyllium plagiosum* was reported to act as GAP for Rab7 (Li *et al*., 2015). TBC1D14 GAP sets the TRAPP (which is a GEF) for ATG9 action which is involved in autophagy (Lamb *et al*., 2016). Recently a Rab11GAP TBC1D12 was shown to modulate neurite outgrowth of PC12 cells (Oguchi *et al*., 2017). In case of tomato RHO/ROP GTPases a total of 9 ROP GAPs were identified as compared to six reported ROPGAPs in *Arabidopsis*. Two RANGAPs were identified in tomato. AtRanGAP1 was used as a marker for the cell division plane in *Arabidopsis* (Xu *et al*., 2008) and plays important role in cellular and developmental function (Boruc *et al*., 2015). The complex analysis of ARF GAP revealed that in tomato there are at least 19 members of ARFGAPs altogether. In *Arabidopsis* a total of 15 members have been reported. The basic set of tomato ARFGAPs consist of 6 member sequences (table3.28). The ARF GAPS with the Ankr(Ankyrin Rich Repeat) and Pleckstrin Homology (PH) domain were also identified and in tomato at present there are 13 members in this group. In *Arabidopsis* a different set of nomenclature for ARF GAP is used as compared to the RabGAPs. The sequences identified with the GAP specific domains related to

ARF in *Arabidopsis* are termed as ARFGAP Domain (AGD) containing proteins with 15 members in *Arabidopsis*. Over expression of AtAGD7 causes relocation of Golgi localized proteins to the ER and inhibits protein trafficking in plant cells (Min *et al*., 2007). This is the first report of ARF GAPs in tomato.

Protein localization prediction

Along with the protein sequence analysis other softwares were used to predict the localization of small GTPases of tomato. The predictor YLoc (Briesemeister *et al*., 2010) and WoLF PSORT (Horton *et al*., 2007) distinguish between 11 different compartments (extracellular, nucleus, Golgi, ER, mitochondrion, plastid, plasma membrane, peroxisome, vacuole, cytosol and cytoskeleton), while TargetP (Emanuelsson *et al*., 2007) and Predotar (Small *et al*., (2004) are highly accepted to distinguish between chloroplast, mitochondria and secretory pathway localization. MitoPred (Guda *et al*., 2004) as mitochondrial specific and ChloroP (Emanuelsson *et al*., 1999) as chloroplast specific localization predictor. YLoc, WoLF PSORT, TargetP, Predotar, MitoPred and ChloroP from SUBA3 localization predictor bundle, are available for the automation of the localization approach and were individually employed for the analysis of small GTPases including RAB, ARF, ARL, SAR, RAN, ROP and their regulators. But the results were not satisfactory and are not included in the thesis. Since majority of the softwares and packages available indicated that the target protein was localized in cytoplasm in case of RAB11 GTPase of tomato and same for ARF, ARL, SAR and ROP. RAN GTPase was shown to localize in the nucleus. Even the results from the SUBA3 were very basic and did not give much detail about the exact prediction of the location of the protein in the endo-membrane compartments.

Molecular and phenotypic characterization of LeRab11a antisense lines.

The seeds from the nine lines of the *LeRab11a* antisense (R3T1) and wild type *Ailsa craig* (AC++) were used for the experiment. The PCR analysis of each of the nine lines named from P1-P9 (24 plants from each line) was carried out. The band of expected size of 0.96 Kb was observed as reported by Lu *et al*., (2001). The lines which satisfied the Mendelian segregation ratio (3:1) were selected. Transgenic line number P4 (100%) and P1 (91.66%) were selected for the present investigation. P1, P2, P3, P4, P5, P6, P7 almost all satisfy the Mendelian

segregation ratio (number of positive plants with transgene were >75% of the tested) and were found homozygous, however P8 had 70.83% positives and P9 showed no transgene. It would be interesting to see if the same phenotypes are observed while testing the other lines except P4 and P1, which were used in the present study). It is also intriguing to check about the number of copy/inserts of the antisense transgene in the transgenic lines. A southern hybridization should be carried out in order to find the number of copies in each independent event and line and what phenotypic effects are observed based on the individual dosage or number of copies of the gene, and to what extent it affects the characteristic phenotype of the LeRab11a antisense tomato plants.

Fig. 7.1 highlights the details of LeRab11a antisense transgenic plants and the phenotypes which were observed by Lu *et al*., (2001) and also in the present investigation. The analysis of the progeny of the primary transformed lines indicated that the phenotypic changes were heritable. Primary transgenic plants exhibited highly branched inflorescence, rumpled leaves, some shoots emanating from rachis and some transgenic plants showed determinate growth as reported by Lu *et al*., (2001). Phenotypes observed in tomato antisense plants might indicate that the phenotype could be due to the disruption in the trafficking carried out by *LeRab11a* gene.

One of the phenotype of *LeRab11a* antisense transgenic plants observed in the present study was different branching patterns with respect to the floral and vegetative meristem developing from the flower cluster/truss and it continued to grow into branches having leaflets, flowers and bearing fruits. Similarly the silencing of *Osrgp1* (*Rab11* related protein) in transgenic tobacco had decreased apical dominance, increased numbers of side shoots and resulted in dwarf plants (Kamada *et al*., 1992) which was resultant effect of high levels of cytokinins (Sano *et al*., 1994) which supports the phenotype observed in transgenic tomato plants. A Class-I TCPs modulated the cytokinins response in leaves and flowers and induced branching and meristematic activity in tomato (Steiner *et al*., 2012, Efroni *et al*., 2013). Thus the possible role of cytokinins can not be ruled out and needs to be probed in Rab11a antisense transgenic tomato lines. The transgenic *Arabidopsis* plants over expressing *Ara2* and *Ara4* genes also led to the pleotropic phenotypes (Aspuria *et al*. 1995).

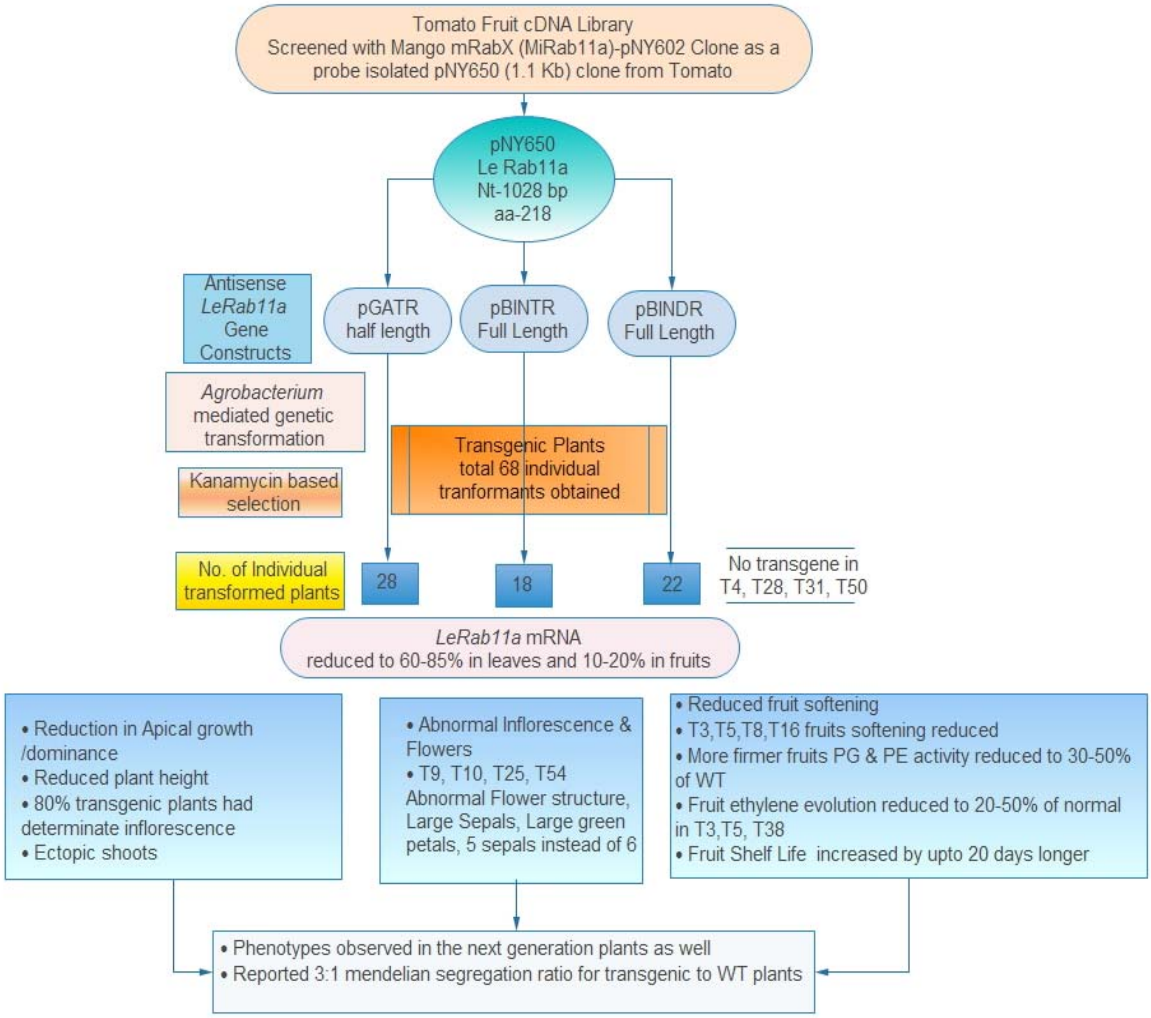


Fig.7.1 Schematic diagram of generation and characteristics of tomato LeRab11a antisense transgenic plants.

The terminating flower gene in tomato was reported by MacAlister *et al*., (2012). Macrocalyx (MC) gene was reportedly involved in the development of sepals in the first whorl and in inflorescence determinacy in tomato (Nakano *et al*., 2012). These results press the need to investigate the expression of the above mentioned genes in wild type and transgenic tomato lines to find and establish the real functionality of the morpho-physiological reason behind the observed phenotype. The repression of floral meristem fate is crucial in shaping tomato inflorescence (Thouet *et al*., 2012), this type of repression might be responsible for the inflorescence observed in the case of *LeRab11a* antisense transgenic plants. This hints towards the possible role played by Rab11a GTPase in the trafficking of such proteins which are involved in shaping the meristem during cell division and cell cycle. The meristem (vegetative or floral) is the active and rapidly growing zone of the plant which requires rapid cell division and constant shunting of

proteins ‘to and fro’ for maintaining the integrity of the cells and tissue for normal development. Since RabA group members have also been reported to be involved in cell cycle progression and cell plate formation, thus pressing the need to study the morpho-physiological role of individual RabA group members in tomato. This needs to be further validated by either constructing a gene regulatory or gene co-expression network or by generating simultaneous expression profiles either by RNAseq or qRT PCR in wild type and transgenic lines. Different kinds of inflorescences were observed in the transgenic plants in present study (chapter 4) and similar phenotypes were also reported by Lu *et al*., (2001). Sometimes simple and compound inflorescence architecture were observed in the Rab11a antisense transgenic tomato lines and these different types of inflorescences have been studied by Lippman *et al*., (2008), Chitwood *et al*., (2013) and Périlleux, *et al*., (2014). The Florigen flowering hormone plays a vital role in the shoot architecture (Shalit *et al*., 2009, Jiang *et al*., 2013, Lifschitz *et al*., 2014, Park *et al*.,2014). It would be interesting to see if there is any reduction or increase in the level of florigen in the antisense line. Xu *et al*., 2015 reported the involvement of arabinosyltransferases as a determinant of shoot meristem size in tomato. Whether trafficking of arabinosyltransferases has had an impact in the antisense transgenic lines, poses an important question? Suppression of leaf meristem also leads to the determinate leaf growth (Alvarez *et al*., 2016). The SINGLE FLOWER TRUSS (SFT) and SELF-PRUNING also play important role in the monopodial and sympodial branching architecture in plants as was observed in case of cotton (McGarry *et al*., 2016). The expression of SFT gene in wild type and transgenic tomato lines should also be investigated. Some of the transgenic tomato plants also exhibited dark green and rumpled leaves, a similar observation was recorded when a putative F-box protein gene SlGID2 was knocked out in tomato resulting in dwarf plants with dark green leaves (Liu *et al*., 2016). SlMBP11 was also reported to be involved in the regulation of plant architecture in tomato (Guo *et al*., 2017). Recently Tal *et al*., (2017) reported that a ‘Kelch repeat protein’ was involved in the coordination of meristem doming and the floral transition by late termination. The above reports highlight the possibilities of the disruption of protein trafficking in the Rab11a antisense transgenic tomato lines resulting in the observed phenotype.

Along with branching pattern and leaves, different types of flower structure was also observed in the present study. Some of the transgenic plants had fused and larger sepals with some showing yellow colour instead of green. Similar results were also reported by Lu *et al*., (2001), where the Rab11a antisense transgenic flowers (12.30% of transformants) had abnormally large petals and sepals and in some cases yellow sepals. Kamada *et al*. (1992) also reported the same phenotypes in antisense transgenic *rgp1* plants where flower buds failed to form flowers and sepals changed to petals. Several floral genes have been reported to show similar kind of phenotype if knocked down. A mutation in TAP3 and the silencing of TM6 resulted in the conversion of stamens into carpels (Quinet *et al*., 2014). The silencing of TPI and SlGLO1 in tomato resulted in aberrant carpelloid stamens (Guo *et al*., 2016) RNAi *TOMATO AGAMOUS-LIKE 1* (*TAGL1*) lines showed pleiotropic phenotypes, ripening inhibition and reduced carpel expansion (Itkin *et al.*, 2009; Vrebalov *et al.*, 2009). The expression profiling of the above discussed genes from the transgenic tomato flowers exhibiting the phenotype should be generated to find if there are differences to possibly hint at the actual mechanism where in the trafficking might have been disrupted in the antisense flowers.

The Rab11a antisense transgenic fruits stayed longer and firmer on the plants and in storage after harvesting as compared to the wild type which was evident from the visual observations recorded as well as the textural analysis undertaken (chapter 4 & 5). The phenotype observed in the T3 transgenic line in the present study supports the results obtained by Lu *et al*., (2001) where the transgenic lines T3, T5, T8 and T16 had reduced fruit softening resulting in higher fruit firmness. The shelf life of the transgenic fruits was around 20 days longer than that of wild type and water content of transgenic fruit was higher than control fruits (Lu *et al*., 2001). Cell wall hydration of *LeRab11a* antisense fruits can also be an interesting parameter to check the effect on the cell wall of red ripe fruits. It would be interesting to note if there are any significant changes in the free radicals (ROS) generated during late ripening stages or the elemental analysis of the cations and anions during the developmental stages of fruit ripening in wild type and transgenic plants.

The consistent weight gain post fertilization until the mature green and breaker stages and a decline in the weight at more than B+50 days could be attributed to the increase in the dry weight initially at early ripening stages. Decline in relative water content of the fruits is important physiological parameters affecting the fruit firmness. The calyx was also firmly attached to fruits and did not show the abscission and was able to hold the fruits even at the very late red ripe stages of fruit ripening. This needs further investigation in terms of measuring the force required to detach the fruits from the calyx and the abscission or zone of separation from where the green calyx is attached to the fruit. This could also be one of the attributes which might not have allowed excessive transpiration or water loss from the fruits, thus helping the transgenic fruits to retain water for longer time and cellular turgor resulting in firmer fruits than wild type at late stages on plants or in storage.

Plant hormones play vital and deciding role in the growth and development. It would be interesting to profile the hormonal interplay of auxin, cytokinins and gibberellin (GA) in the transgenic plants to check if there was any disruption in signalling cascades which led to the phenotype observed in the plants. These three hormones play a vital role in the growth and development of plant especially in the determining the height, branching and pattern of flowering in plants which was fund to be deviating from the normal wild types. This raises an important question and possibly hints that there might be perturbation of trafficking in the antisense tomato lines of the proteins leading to an altered biosynthetic and the signalling pathway or cascades resulting in the different growth, branching pattern and inflorescence development. The *procera* tomato mutant had altered flowers with one additional petal, sepal, stamen and carpel at each of the four whorls (Carrera *et al*., 2012, Livne *et al*., 2015). GA also modulates the activity of SLARF7 (Auxin response factor7). SlARF2a plays a negative role in mediating axillary shoot formation (Xu *et al*., 2016). Auxin and LANCEOLATE affect leaf shape in tomato (Ben-Gera and Ori, 2012). The interaction between auxin, gibberellin (GA) and cytokinin (CK) plays important role in tomato leaf development (Shwartz *et al*., 2016). Cytokinin also regulates compound leaf development in tomato (Shani *et al*., 2010). These reports hints at the normal mechanism of growth and development where the trafficking of the above

mentioned hormones and proteins involved in their biosynthesis is properly regulated but in contrary the balance was supposedly distorted in case of the Rab11a antisense tomato lines. Thus inhibition the trafficking carried out by Rab11a and the other group members play an important role in the normal growth and development of plants including height, branching, inflorescence and flower development.

It would be interesting to see the co-expression of the other ripening related genes and transcription factors governing them with the Rab GTPase group of genes at different developmental stages of fruit ripening. A carbohydrate array with the available antibodies of cell wall hydrolases and a co-expression network of other proteins involved in the vesicle trafficking during the peak stages of ripening can yield insight into the intricate mechanism of trafficking. Other group of cell wall associated proteins (fig. 7.2) at different fruit ripening and developmental stages with Rab GTPases can be used to generate expression pattern to decipher the temporal distribution of these genes in different tissues in tomato fruit development highlighting their role in fruit ripening and softening.

Textural analysis of tomato fruits

The textural analysis by compression and penetration test revealed that there is a pattern of delayed softening in transgenic fruits, which supports the results obtained by Lu *et al*., 2001, Phan *et al*., 2007 and Lunn *et al*., 2013. Delayed pattern of fruit softening of the LeRab11 antisense fruits and the retention of the skin tightly to the calyx and less susceptibility to the fungal diseases at the last stages of fruit ripening is remarkable as compared to the wild type which most often becomes susceptible to the loss of firmness, loss of turgor and an absolute degradation of the fruit quality and shelf life at later stages beyond 70-80 days after the onset of breaker stage. The shelf life of fruit is defined as the period during which a stored product remains suitable for consumption and is normally determined by the degree of softening, shrivelling and rotting of fruit. Fruits which soften greatly as they ripe acquire a melting texture and usually having a short postharvest life (e.g. strawberry, peach, apricot, tomato) and those which soften moderately retain a crisp texture and have a long storage life (e.g. apple, pear, watermelon).

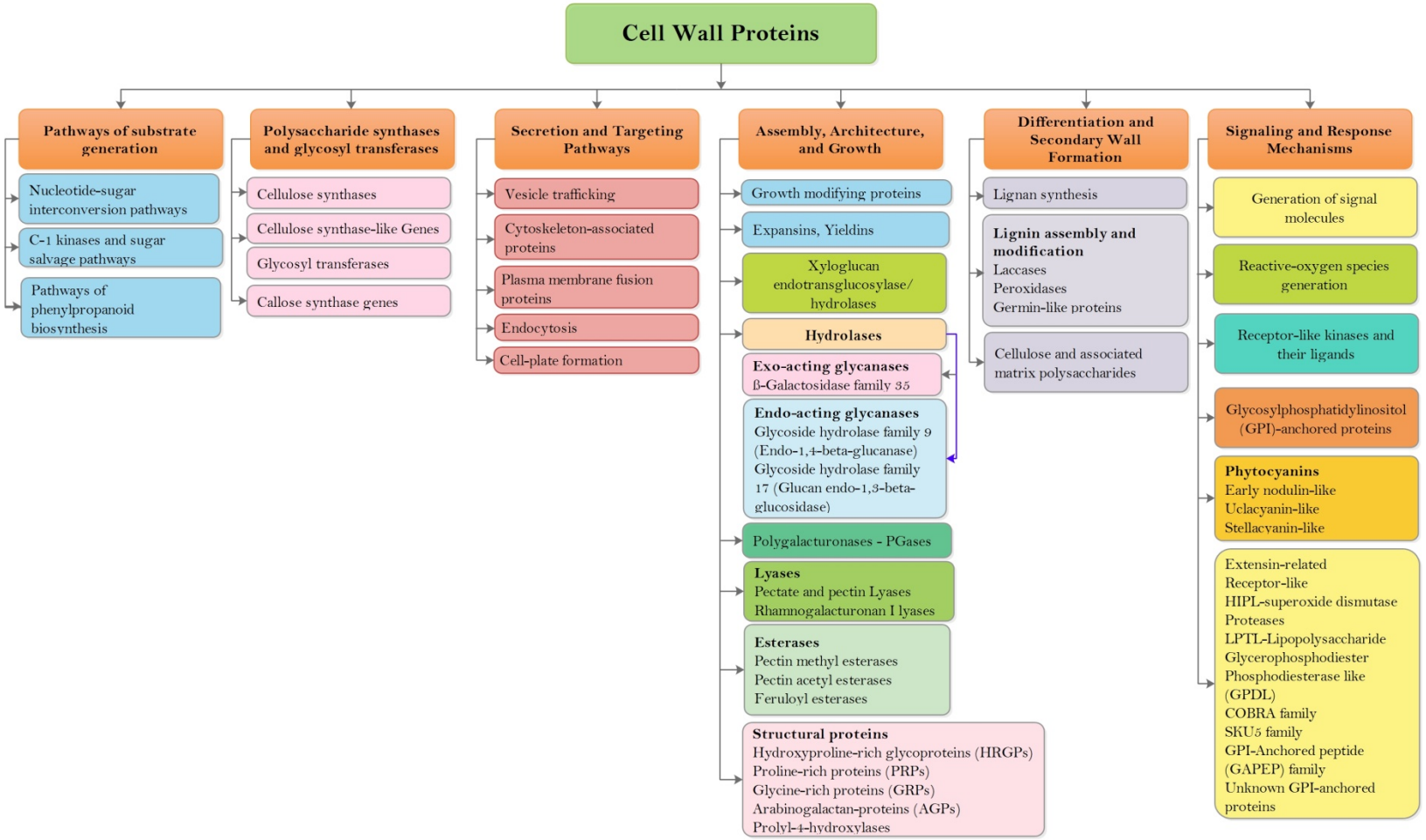


Fig. 7.2 Cell wall Proteins: Different classes of cell wall proteins involved in the substrate pathway generation, polysaccharide synthesis, secretion and targeting, cell wall assembly, architecture and growth, differentiation and formation of secondary cell wall components and other involved in signalling.

Firmness can be defined as compressibility or the force required to deform the surface of fruit (Brookfield *et al*., 2011). Texture is a complex sensory attribute. Cellular turgor contributes to fruit firmness and textural properties which is maintained by water and solute homeostasis within fruit and is also affected by its distribution within the cell and in the cell wall (Seymour *et al.*, 2013). Selection of probe is important, a blunt end probe was used in the present study, time and force required to compress or penetrate the fruit gives a fair measure of the resistance provided by the fruit against the probe which can be attributed to the skin strength (cuticle), tissue integrity at different developmental stages of fruit ripening.

Trafficking of the genes listed in fig.7.2 might be disrupted in the antisense tomato lines as was reported by Lunn *et al*., (2015) in *Arabidopsis* where the knockout mutants of different RabA class of GTPases had an impact on the saccharification efficiency in stem tissue because of altered cell wall composition. The plant secretory pathway are crucial for building and maintaining the plant cell wall and for the trafficking of cell wall polysaccharides and glycoproteins (Kim and Brandizzi, 2014, 2016; Paiva, 2016; van de Meene *et al*., 2017; Poulsen *et al*., 2017).

The disassembly of polysaccharide networks in the primary cell wall and middle lamella leads to fruit softening (Rose *et al*., 2003; Brummell, 2006). The cell wall plays an important role in maintaining the integrity of the fruit, The cellulose synthase (CESA) proteins play important role in cell wall assembly, secondary cell wall formation and cell expansion (Bashline *et al*., 2014; McFarlane *et al*., 2014; Schwerdt *et al*., 2015; Tavares *et al*., 2015) and are also important for development of floral and fruit abscission zones during fruit development (Tsuchiya *et al*., 2015). It would be interesting to find the expression pattern of these enzymes in the antisense line.

The Xyloglucan xyloglucosyl transferase/endohydrolase (*XTH*) transgenic fruits had a more than fourfold increase in *XET* activity associated with reduced xyloglucan depolymerisation and reduced fruit softening (Miedes *et al.*, 2010). Recently AtXTH3 was reported to catalyse the covalent cross linking between cellulose and oligosaccharide which suggest XTH's role in maintaining the cell wall integrity (Minoia *et al*., 2016; Shinohara *et al*., 2017). It would be interesting to see if there is a difference of XTH turnover in LeRab11a antisense lines. The transgenic fruit with silenced *SlExp1* retained a firmer texture than the wild-type

fruit (Rose *et al.*, 1997; Minoia *et al*., 2016). Down-regulation of *Exp1* and *PG* led to firmer fruit texture and higher cellular integrity during longer storage (Powell *et al.*, 2003). Similar results were obtained by Dal Santo *et al*., (2013) for expansin genes in grapes. In contrast to silencing Perini *et al*., (2017) reported that the over-expression of the carbohydrate binding module from Sl-EXP1 (CBM-Sl-EXP1) delayed softening and fruits were less susceptible to *Botrytis cinerea*.

The over-expression of Delila (Del) and Rosea1 (Ros1) led to development of purple tomato with improved shelf life compared to wild-type. The purple tomato fruits lost the integrity and collapsed after 10 weeks of storage as compared to 5 weeks in case of wild type fruits. The textural analysis revealed that wild type red fruits had 50% lower firmness as compared to breaker stage after 2 weeks of storage at 18°C, whereas the same reduction in firmness was reached only after 5 weeks of storage of purple fruit (Zhang *et al*., 2013). This highlighted the role of anthocyanins in fruit firmness.

The results obtained in the present investigation showed that the transgenic LeRab11a antisense fruits retained water longer than the wild type as evident from the visual observation and the textural analysis by compression and penetration. Similar results were reported by Lu *et al*., (2001) in context of the firmness and water retention by the transgenic tomato fruits. A loss of turgor pressure is generally observed during fruit ripening due to regulated accumulation of apoplastic solutes (Wada *et al*., 2009). Transpirational water loss through the cuticle could also be involved, especially in fruits with thick and well developed cuticles, such as tomato (Saladie´ *et al*., 2007; Lara *et al*., 2014; Yeats and Rose, 2013; Domínguez *et al*., 2015; Fernández *et al*., 2016; Segado *et al*., 2016). GPAT6 from tomato plays a central role in fruit cutin biosynthesis (Petit *et al*., 2016). Philippe *et al*., (2016) reported different mechanisms of polymerization by ester cross link in the cutin synthase tomato mutants. Recently Fernandez-Moreno *et al*., (2017) studied the QTLs to identify the candidate genes for fruit cuticular lipid in the *Solanum pennellii* ILs. A study of cuticle by microscopy will be an interesting parameter to find out the skin strength and the retention capacity of the transgenic fruits.

Phan *et al*., (2007) generated antisense transgenic tomato plants with *pmeu1* which is an early expressed PME in tomato fruit. The analysis of PMEU1 in *LeRab11a* antisense fruits suggested that *LeRab11a* was not involved in the

trafficking of PMEU1 to the cell wall. Interestingly, tomato fruit with reduced *PME* expression also exhibited a reduction in fruit blossom end rot, a calcium-associated fruit disorder (de Freitas *et al.*, 2012). An increase in the PME expression leads to a corresponding increase in the calcium which is bound to the cell wall which in turn leads to less availability of calcium for other cellular functions and thus leads to increased susceptibility to the blossom end rot in tomato. The imbalance in the cation and anions also leads to the electrolyte leakage of the plasma membrane which does impact the water distribution. Cellular organization is different in the pericarp and the locular region where seeds are covered in a jelly like dense substance and the pectin methyl-esterification occurs differently in each tissue. Pectin and calcium cross-linking and their subsequent interaction results in a high-density cell wall structure that plays an important role in maintaining fruit firmness. Pectin degradation is associated with fruit softening, and it is thought to increase in the inner fruit tissues, mesocarp, endocarp and locular tissue. Septum connects the pericarp, placenta and locules, a reduction in the septum firmness also affects fruit softening. Pectin disassembly has been reported to be linked with the fruit softening (Hyodo *et al*., 2013; Paniagua *et al*., 2014; Segonne *et al*., 2014; Sénéchal *et al.,* 2015; Fasoli *et al*.,2016; Guillon *et al*., 2017). Inhibition of vesicle trafficking in antisense tomato was associated with reduced pectin levels resulting in reduced softening (Lunn *et al*., 2013). In *Arabidopsis* cell wall pectic arabinans were shown to influence the mechanical properties in the inflorescence (Verhertbruggen *et al*., 2013). The lower cell wall pectin solubilisation and loss of galactose during early fruit development in apple was correlated with slower softening of fruits (Ng *et al*., 2015). Recently Lionetti *et al*., (2017) reported that three Pectin Methylesterase inhibitors were involved in the resistance to Botrytis in *Arabidopsis*. It will be interesting to find if there is altered expression or trafficking of these enzymes at later stages of fruit ripening in case of transgenic fruits and if they are also involved in the reduced susceptibility of fruits to gray mould fungus. The pectate lyase (PL) antisense strawberry fruits showed decrease in ripening associated firmness during fruit development (Santiago- Doménech *et al.*, 2008). The PL::RNAi lines of tomato also showed enhanced shelf life and retained firm texture as compared to wild type (Uluisik *et al*., 2016). Recently Yang *et al*., (2017) also reported that silencing of pectate lyase (SlPL) enhanced

fruit firmness and shelf life as well as was less susceptible to botrytis. The suppression β-mannosidase and β-D-*N*acetylhexosaminidase led to reduced softening and enhanced shelf-life whereas; their ectopic expression caused excessive softening of the transgenic fruit (Meli *et al.*, 2010).

Silencing of genes involved in ABA biosynthesis and signalling led to ripening inhibition in strawberry and reduced rates of cell wall dissolution in tomato leading to improved shelf-life (Chai *et al.*, 2011; Jia *et al.*, 2011; Sun *et al.*, 2012). It would be interesting to see if the trafficking of proteins involved in the ABA biosynthesis were affected in the transgenic tomato lines which showed delayed softening. Sobolev *et al*., (2014) generated a homozygous transgenic tomato (2AS-AS) that exhibits reduced ethylene production as a result of impaired expression of *LeACS2* gene by its antisense RNA and fruits had a longer shelf life. In case of atv (atroviolacea), Aft/Aft atv/atv tomato had enhanced shelf life where the 100% fruits softened after 70 days of storage at 17°C as compared to 42 days of storage for Ailsa Craig fruits (Jones *et al*., 2003; Al-sane *et al*., 2011). These reports on delayed softening and enhanced shelf life in transgenic tomato also emphasises their potential role in Rab11a related trafficking in transgenic fruits. A co-expression network of RabA GTPases with the genes involved in the ethylene biosynthesis as well as the regulators of ethylene biosynthesis could also yield insight into the mechanism of delayed softening in transgenic tomato lines.

Over expression of MADS-box gene SlFYFL in tomato resulted in delayed senescence, fruit ripening and abscission (Xie *et al*., 2014). Similarly over-expression of SlNAC1 in tomato altered fruit pigmentation and softening (Ma *et al*., 2014) whereas its suppression delayed fruit ripening (Meng *et al*., 2016). The transgenic plants over expressing polycomb group (Pcg) protein MULTICOPY SUPPRESSOR OF IRA1 (SlMSI1) had abnormal non ripening fruits whereas, its suppression enhanced fruit ripening (Liu *et al*., 2016). The apple SEPALLATA and peach *SEPALLATA* (PrupeSEP1) gene was reported to regulate ripening and softening (Ireland *et al*., 2013; Li *et al*., 2017). It would be interesting to see the co-expression of Rab GTPases and these genes during different developmental stages of fruit ripening which may yield insight into the differential trafficking in antisense tomato fruits which might be involved in delayed softening.

Expression profiles of Rab GTPases in tomato

The primers were designed by Primer3 software for Rab11/A GTPases in tomato and were validated by BLAST in the TIGR database initially to check 100% hit for the individual target genes and were also validated by RT-PCR (fig.6.1). The typical results of the primer designing as described in section 6.3.2 and rest of the results of other primer pairs for Rab11/A GTPases are in supplementary files. Since Rab11/A group members are closely related, it was difficult to design primers initially and several rounds of careful inspection and analysis were required to finalise the primer pairs which did not overlap and hit other closely related Rab11/A group members to ensure that individual Rab11/A GTPases were amplified in order to avoid the spurious duplication which could have generated false expression profile for the genes in question. With the release of tomato whole genome sequence version ITAG2.4 in SGN database, the primers for Rab11/A GTPase group were also screened and tested by the *in silico* PCR functionality of SGN database. The primer pairs designed for the Rab11/A GTPases from the TIGR database were used to screen the SGN database for cross validation with the SGN-Unigenes and corresponding SolycIDs. The issue of discrepancy in both databases was resolved by employing the reverse BLASTs to associate SGN-Unigenes and SolycIDs of Rab GTPases which eventually gave satisfactory results along with the expected band size (table 6.3).

Systematic validation and normalization of qPCR data is a must and careful selection of the housekeeping reference genes is also important as reported by Huggett *et al*., 2005; Gutierrez, 2008; Guenin *et al*., 2009; Dekkers *et al*., 2012; and Monaco *et al* 2010. The DDCT method (Livak and Schmittgen, 2001) was used for analysing the qPCR data in the present investigation. Gene expression patterns during fruit development and maturation in pear (Zhang[a] *et al*., 2016) and tomato (Zhang[b] *et al*., 2016; Fernandez *et al*., 2017) provides insights into early fruit development processes crucial for ripening and softening.

The qRT-PCR results were used to generate the expression profile to find out which genes belonging to the Rab11/A groups were up or down regulated in wild type and transgenic *LeRab11a* lines to find the putative candidate genes. The three putative candidate genes that were expressed highly are TC199565 until mature green stage and the others are TC207026, TC208692 and TC202127. The target

LeRab11a used for the generation of antisense transgenic lines 'TC199565' had an increased expression pattern from 7 DPA until mature green and breaker stages but after breaker and Breaker+10, the expression of TC199565 started to decline, similar results were also reported by Lu *et al*., (2001). The expression of LeRab11a GTPase was considerably down-regulated in the transgenic fruits in the present investigation which supports the findings of Lu *et al*., (2001) and Lunn *et al*., (2013). Rab11a/A1a group members play an important role in cell division, cell plate formation and mobilization of the proteins to the membrane in forming the new cell wall for re-localization of the cell wall material to the new site of synthesis and their trafficking (Preuss *et al.,*2004; Chow *et al.,*2008; Szumlanski and Nielsen, 2009; Bottanelli *et al.,* 2011; Kang *et al.,* 2011; Feraru *et al.,* 2012; Asaoka *et al.,* 2013; Choi *et al.,*2013 Berson *et al.,* 2014; Kirchhelle *et al.,*2016). All these indicate the important role played by RabA1a GTPases and as anticipated during the initial developmental phase of fruit growth it showed an increase in expression where the cell division is taking place until the fruit attain the mature green stage. When the expansion phase ceases, the cell and tissue undergo considerable remodelling which leads to increase in the cell size instead of cell number. Changes in the texture, aroma pigmentation, acidity in the locular juices all these conglomerate and myriads of changes need efficient trafficking where the fruit attains the required changes in colour from green, reddish orange at breaker stage and finally red colour at ripe stage. The turgidly and the skin strength is required to hold and maintain the cellular and tissue integrity. Once the fruit passes the red ripe stage its starts to collapse, undergoing an evolutionarily conserved process of decline in water retention, loss in turgor and firmness, collapse of tissues and development of abscission zones for fruit drops for dissemination of seeds. The expression of RabA1a declined post red ripe stages as was observed in the present study. During the last stages i.e. post red ripe stages the fruit cell wall undergo considerable changes where the cell wall needs to be broken down. The dissolution of the cell wall middle lamella occurs. The proteins/enzymes e.g. cell wall hydrolases, required to carry out the process needs to trafficked at the right time in right proportion (Lycett, 2008). This task is carried out by RabA GTPases to sustain the normal cycle of fruit growth, development, ripening and softening. RABA2, RABA1c, RABA1d, and RABA1e were shown to be recruited at the cell plate during cytokinesis in *Arabidopsis* root

tips (Chow *et al*., 2008, Asaoka, *et al*., 2012, Qi *et al*., 2013, Oda *et al*., 2014, Berson *et al*., 2014). The expression of other RabA GTPase varied in tomato during the fruit development, as was observed in the present study which indicated that each individual paralogs have evolved a different function for trafficking of different proteins. It would be interesting to investigate the role of other members of RabA group members in tomato fruit ripening and softening.

The expression profiles were also generated by analyzing the raw data from the TED database (Fei *et al*., 2006) for wild type tomato (AC++) by downloading the raw files for which the expression data was available and could be assigned to the latest SolycIds. The expression profiles of different available Rab GTPases from class1 to class18 were generated. The results of the analysis presented in the thesis showed that the expression pattern of Rab1/D in AC++ was higher at the breaker stages as compared to other stages of fruit ripening and declined after the breaker stages. However, Solyc08g078070 showed significant increase in the expression, where the expression goes on increasing from IMG to MG and from MG to Breaker stage and then significantly decreased at red ripe stage of fruit development. These results follow the same pattern with that of RabA1a. Since Rab1/D is involved in the trafficking from ER to Golgi, the results indicate that during the initial growth and expansion of fruits the proteins synthesized at ER are rapidly trafficked from ER to Golgi for the post translational modification and are trafficked to their destination from TGN to the target site which is in fact carried out by other Rab GTPases e.g RabA. Thus the above results do indicate the simultaneous and parallel expression pattern required for trafficking during the early fruit development until the fruit reaches mature green stage. Loraine *et al*., (1996) isolated three tomato cDNA (LeRab1A, 1B, and 1C), out of theses three LeRab1A was found to be more abundant in ripening fruit which in contrast to the results obtained in the present study and is worth investigating.

The expression pattern for Rab2/B GTPase for the three genes were almost similar except for 'Solyc06g060210' gene which was down regulated at IMG and MG stages as compared to the other three group members. Its expression slightly increases only at breaker stage but then again declined like other Rab2 GTPases. Solyc02g036450 and Solyc02g081380 followed almost same pattern. Their expression is fairly similar at the IMG stage to MG stage and there was no significant rise from MG to Breaker stage, but the expression of both genes

declined at the red ripe stages. The expression profiles of Rab2/B which is involved in the trafficking from ER to Golgi, also follows the same pattern as that of Rab1/D but there is no remarkable increase or decrease across the different stages of fruit ripening. A comparative study of Rab1/D and Rab2/B needs to be carried out to find which proteins are being trafficked from ER to Golgi.

In case of Rab5/F GTPases, the gene 'Solyc02g069370' showed a gradual increase right form the IMG to MG stage and from MG to Breaker stage, but there was no decline in the expression of this gene from Breaker to RR stage of fruits as compared to the other two Rab5 GTPase which decline at red ripe stage as well as with Rab1/D and Rab2/B. But in contrast to the expression of Solyc02g069370' the other two Rab5/F i.e. Solyc02g036450 and Solyc02g081380 decreased significantly at the red ripe stage after breaker which is worth investigating. Rab5/F GTPases regulate trafficking form PVC to vacuoles which hints at the possible role they play in the trafficking of proteins involved in the cellular homeostasis, and maintenance of vacuolar pH until red ripe stages. No significant differences were found in case of Rab6/H GTPases, as all genes more or less followed the same pattern. The Rab6/H GTPase group is involved in the trafficking from ER to Golgi to TGN. As compared the expression patterns of other Rab GTPases during fruit ripening the Rab6/H group maintains fairly a stable expression pattern across all stages. The Rab7/G GTPase gene with SolycId- 'Solyc04g072060' was expressed significantly lower as compared to Solyc01g109520 and Solyc03g120750 throughout the developmental stages. Rab7/G GTPases regulate trafficking from PM to vacuole and are involved largely in endocytosis and most of the reports indicate that they are involved in the abiotic stress tolerance and maintenance of cellular homeostasis. In case of Rab8/E GTPases which are involved in the Golgi to PM trafficking, the expression of Solyc06g005350 was significantly down regulated as compared to the other three Rab8/E GTPases. Its expression decreased from IMG to MG and it decreased rapidly from MG to breaker stage but remained stable with no significant change from the breaker to RR stage. In contrast to the expression of the other two Rab8/E GTPases the gene Solyc09g010370 and Solyc10g086350 showed a gradual increase from IMG to MG stage but the expression slightly declined from MG to breaker. It would be interesting to see which proteins are

trafficked by this GTPase and what role it plays in the tomato fruit development and ripening.

The expression of the Rab18/C gene-Solyc04g064510 was up-regulated as compared to the other two Rab18 GTPases. The two Rab18/C GTPase *viz.* Solyc09g008460 and Solyc10g086310 showed decrease in expression form the IMG to MG and also from Breaker to RR stage. Rab18/C GTPases were reported to be involved in the trafficking to endosomes and were largely involved in the trafficking of lipid droplets. This class of GTPases is an interesting target for the future investigation as it raises question whether it's involved in the maintenance of phospholipid bilayer of PM or the membranes of other endosomal compartments.

In case of our target LeRab11a GTPase with SolycId Solyc07g055290, its expression increased from IMG to MG and started to decline at the RR stages as was observed in qRT-PCR results in the present investigation and similar results were also reported by Lu *et al*., (2001) and Lunn *et al* ., (2013). Northern bolts and qRT PCR analysis of endogenous *LeRab11a* in wild type plants by Lu *et al*., (2001) at different developmental stages of tomato fruit ripening at 15 DPA, 30DPA, 45 DPA, Breaker, Breaker+5 and Breaker+10 showed that the expression of *LeRab11a* was higher at early stages of fruit ripening and declined at later red ripe stages as was observed in the present investigation. The above two reports are in agreement with the results obtained in this study and describes the possible role of RabA1a GTPase as discusses earlier. The down regulated Rab11/A GTPase genes include Solyc03g006270 (RabA1) and Solyc03g079900 (RabA2) which were down regulated in the IMG, MG, breaker and red ripe stage. But the expression increased inversely in case of low expression. The above results raise an interesting question about their possible role and involvement in trafficking at different fruit ripening stages as they significantly deviate from the expression pattern usually observed in case of other paralogs of tomato RabA GTPase group members RabA1 and RabA2. The expression pattern shows that may be the proteins which are required at the initial fruit growth stages are trafficked at IMG stage and thereafter the expression of both these genes are significantly down regulated. It would be interesting to see what effect they have on fruit ripening if they are knocked out and a parallel study needs to be undertaken to find which proteins are trafficked by them. The expression pattern of another RabA1 group

member Solyc02g072180 was interesting as it constantly decreased from IMG to MG; MG to breaker and its declined remarkably at RR stage, which is worth investigating. This analysis helped in gaining a fair idea about the expression pattern of different Rab11/A GTPases at different developmental stages of fruit ripening. A study to investigate the sub-cellular localization and a possible vesicle proteomic study at different developmental stages of fruit ripening should be undertaken to find the proteins and enzymes trafficked by the individual RabA GTPases group members.

The expression profiles of the small GTPases have also been reported in other fruits as well. The expression analysis of the *albedo* and *flavedo* tissues between puffed and control citrus fruits found that SAR1, RABA1, RABC1, RABF1, RAB18B and RABA3 were up-regulated along with some genes related to calcium regulation (Singh *et al*., 2014). Seven RabGTPase were up-regulated at 217 DAP in the olive abscission zone, i.e. RABG3A, RABG3D, ARA4, ARA6, RABA2B, RABA2A/RAB11C and RABC2A (Gil-Amado and Gomez-Jimenez, 2013). The above report also hints at the possible involvement of RabA GTPases and other tomato GTPases in the development of the abscission zone, which highlights the results of the present study where the LeRab11a antisense transgenic red ripe fruits stayed longer and were firmly attached to the calyx of the truss. The above report in olive is also suggestive of the involvement of Rab7/G and Rab18/C in the development of abscission zone, which is worth investigating. The RNA-seq analysis followed by qRT-PCR in channel catfish post bacterial infection revealed up-regulation of at least 10 Rab GTPase genes *viz*., Rab3a, Rab4a, Rab4b, Rab5a, Rab5c, Rab7a, Rab9a, Rab11a, Rab11b and Rab33a (Wang *et al*., 2014), thus providing new insight into the links between Rab GTPases and disease development. A comparative study of the fruits infected with *Botrytis* in wild type and transgenic line can reveal the differential expression profiles of the Rab GTPases in tomato to identify the genes involved in the possible tolerance of tomato fruits to the gray mould as was observed in the present study. The expression profiles were also reported in peach (Falchi *et al*., 2010), grapes (Abbal *et al*., 2008), tomato (Lunn *et al*., 2013) and wheat (Tyler *et al*., 2015). Falichi *et al*., 2010 reported that PpRABA1-2, PpRABA4, PpRABA5, PpRABA6, PpRABB1, PpRABC1, PpRABE1, PpRABF2, and PpRABG3 showed differential expression pattern whereas, the expression of PpRABA1-1,

PpRABA2, PpRABD2-1, PpRABD2-2, and PpRABC2 exhibited two transient up-regulations, at 72–86 DAA and 86–107 DAA. Altogether the results of the present study and other reports highlight the importance of these genes in the developmental regulation of fruit ripening and possible role in fungal disease tolerance. In the quantitative analysis of nuclear proteome of wild type tomato Ailsa Craig, RanA1 (Solyc05g023800) was reported to be up regulated at the breaker and orange stage but interestingly was found to be down regulated at the MG and RR stage along with ARL (Solyc03g043960) GTPase (Wang *et al*., 2014). The above report also presses the need to generate the expression profile of other group of small GTPases in tomato like ARF, ARL, SAR, RAN and ROP and to investigate their possible role in fruit ripening and softening.

Along with the data retrieval for the gene expression in wild type *Ailsa craig* (AC++), two more datasets were also analysed to find the expression pattern of Rab11/A GTPases in Heinz cultivar of tomato and of *Solanum pimpenifolium* from the TomExpress (Zouine, 2017). In case of Heinz cultivar, the genes with SolycIds- Solyc03g064020 (RabA4), Solyc05g052900 (RabA1a) and Solyc10g079030 (RabA4) were down regulated in 1cm Heinz fruits. The genes Solyc03g006270 (RabA1), Solyc03g079900 (RabA2) and Solyc11g010100 (RabA4) were found to be down regulated at breaker stage. In case of Heinz cultivar the RabA4 group members were found to be mostly down regulated at the initial developmental stages. This needs to be further investigated the role of the down regulated RabA4 group members. The results presented in the thesis also presses the need to generate the expression profiles of individual genes in comparison to the other co-expressed genes identified through network analysis to gain more clearer understanding and to identify the potential candidate genes. RabA3 and RabA6 with just one member each in RabA group also poses an interesting question, since the numbers of paralogs in RabA group members of other class are higher, which probably hints at the diversified functionality with respect to the trafficking needs of the cells. Hence the investigation of RabA3 and RabA6 genes should be carried out to find the proteins being trafficked by them. The expression for 15 of Rab11 GTPase genes in *S. pimpenifolium* were also retrieved at three different developmental stages including IMG, Breaker and Breaker +5. In most cases the expression gradually declined from MG to Breaker and from breaker to the B+5, with the exception of the three genes

Solyc03g006270 (RabA1), Solyc03g079900 (RabA2), Solyc05g052900 (RabA2) with erratic pattern of expression which were also supposedly down regulated in all three stages. This raises an interesting question with respect to the down regulation of RabA4 group members in Heinz as compared to the RabA2 group members in *S. pimpenifolium* which needs further investigation as the pattern observed is specific to the cultivars, growth habits or the genotypic backgrounds. Along with this the expression pattern of *LeRab11a* GTPase in top, middle and bottom part of wild type (AC++) tomato fruit at six different developmental stages IMG10, IMG20, MG, Brk, B+5 and B+10 days were also retrieved. The comparatively higher expression in the top section of tomato fruit is reasonable since the top section of the tomato fruit is attached to the calyx and plant, and the fruit sources all of its nutrients from the top section. The change in colour at breaker and post breaker stage starts from the bottom section of the fruits. The higher expression in top section may well elucidate the necessity of the Rab11a GTPase to traffic more proteins during the cell division and cell expansion phase. It would be interesting to generate expression profiles of different GTPase for the individual sections of tomato mesocarp and endocarp, layers of pericarp, as well as the cuticle and columella to hunt for the differences. It would also be interesting to find the expression patterns of other small GTPases identified in the present investigation including ARF, ARL, SAR, RAN, ROP the regulators of RAB GTPases like RabGDI, GEF, GGTase and RabGAP in these sections of fruit.

This highlights the possibility of generating a temporal and spatial expression pattern of LeRab11 group of GTPases and other group of Rab GTPases as well as other small GTPases and their regulators in fruit tissues at different developmental stages of ripening. Single cell analysis is also paving its way to the future. It would be interesting to see if the technology can be used for investigating cellular distribution of GTPases within different tissues of fruits. Astola *et al*., (2014) analysed the gene network underlying the branching of tomato inflorescence. Arhondakis *et al*., (2016) carried out the *in silico* transcriptional regulatory networks involved in tomato fruit ripening and Fatima *et al*., (2016) reported the fruit metabolite networks in tomato. The metabolic profiling of the different developmental stages in the wild type AC++ and the antisense transgenic fruits could also yield more interesting results.

Thus to conclude the present investigation led to the identification of different small GTPases and their regulators in tomato. The expression profiles of Rab GTPases also highlight the differential expression patterns observed across the different developmental stages along with the phenotypic and textural analysis of the wild type and transgenic fruits. These results met the underlying objective of the present investigation and were also able to highlight their potential to carry out further investigation based on the present study to better understand the role of Rab and other small GTPases in the intracellular membrane trafficking in tomato fruit ripening.

8. Conclusion and Future work

Conclusion

The results of *in silico* analysis from the present investigation were able to identify and classify the small GTPases in tomato including Rab GTPases, RAN, ROP, ARF, ARL, SAR and their regulators GEF, GDI and GAPs. The information about the genes was updated throughout the course of the present investigation right from the TIGR DFCI TGI release11,12 and 13, SGN-Unigene Build 1 and 2 as well the latest release of tomato whole genome sequence database at SGN from SGN-ITAG 1 to ITAG 2.4. After the final analysis a total of 55 Rab GTPases were grouped into eight different sub classes with five Rab1/D, five Rab2/B, four Rab5/F, three Rab6/H, four Rab7/G, five Rab8/E, twenty five Rab11/A and four Rab18/C. Solyc Rab11/A was further classified into 6 sub groups from A1 to A6. The results of classification led to identification of seven RabA1, six RabA2, one RabA3, five RabA4, five RabA5 and one RabA6. The phylogenetic analysis of the tomato Rab GTPases and a comparative analysis with Arabidopsis Rab GTPase confirmed the groups and classes. Similarly four RAN, ten ROP, twelve ARF, four SAR, six ARL GTPases were identified. In case of ARF GTPases seven ARFA, four ARFB, and one ARF C was found. The regulators of small GTPases identified in tomato had one RAB GEF, two ROP GEF, four RABGDI and five ROP GDI, twenty two RabGAP, two RAN GAP, nine ROP GAP and nineteen ARF GAP. Comparative phylogenetic analysis of all the above with Arabidopsis genes confirmed the classification. The molecular and phenotypic characterization confirmed the presence of antisense *LeRab11a* transgene and the phenotypes were heritable. The textural analysis gave the evidence that the transgenic fruits were firmer than wild type at the later stages of fruit ripening and had enhanced shelf life and reduced susceptibility to the fungal diseases. The relative expression profile generated by qPCR also showed the pattern of differential expression of Rab11 group of GTPases in wild type and transgenic fruits. Along with this the latest freely available expression database from SGN-TED, TomatoExpress, TomNET were also used to generate the expression profiles of other classes of Rab GTPases during different developmental stages not only in

wild type Ailsa Craig (AC++) but also in Heinz cultivar as well as in *Solanum pimpinellifolium*.

Future work

The comprehensive analysis of small GTPases in tomato along with their expression profile opens up an avenue to find the target GTPases of different groups which show remarkably differential expression pattern in wild type fruits at different developmental stages especially at post breaker and late red ripe stages. The candidate genes could be silenced by reverse or forward genetics *e.g.* by stable or transient transformation with dominant negative mutations or antisense/RNAi constructs. The CRISPER/CAS9 technology also holds promise and it can be utilised for editing the tomato genome directly with specific target genes involved in the membrane trafficking (Mao *et al*., 2013; Ulisik *et al* 2016; Ueta *et al*., 2017; Cermak, *et al*., 2017).

Using VIGS for the candidate genes found in this study could well give more information about their role. Fruit softening has been characterized and have shown promising results as the fruits remain firmer for a long time in transgenic plants as compared to the wild type plants. But still the reason for the skin strength and the tight attachment of the calyx to the antisense plant's fruits is unknown. It would be interesting to characterise the location of Rab proteins and of the vesicle cargoes in wild type and mutant systems. This might involve labelling of cell sections or use of GFP protein fusions to come to a more concrete conclusion about localization and the role of Rab GTPases in trafficking of the enzymes which are involved in the cell wall modification. The antibodies for the polysaccharides could be used along with the GFP or RFP protein fusion complex of Rab GTPase to exactly find which Rabs are involved in trafficking to specific sites or specific target membranes. The multicolour marker sets to tag the individual membrane compartments or vesicles also provides a valuable tool for co-localization and targeting studies (Geldner *et al*., 2009; Moore and Murphy (2009). Dynamic nature of trafficking makes identification of the cargo moving in different vesicles particularly challenging. The emerging technologies of chemical genomics, organelle proteomics, cell wall analysis using OLIMP, the use of polysaccharide antibodies and tagged substrates, and the use of fluorescent tagged screens in combination with next-

generation sequencing are promising avenues for dissecting several aspects of cell wall biosynthesis and polysaccharide transport (Obel *et al*. 2009; Drakakaki *et al*. 2011; Anderson *et al*. 2012; Avci *et al*. 2012; Drakakaki *et al*. 2012; Nikolovski *et al*. 2012; Parsons *et al*. 2012a; Sparkes and Brandizzi 2012). Recently fluorescent fingerprinting method was also employed to study pectic polysaccharides during fruit ripening (Airianah *et al*., 2016).

Imaging ellipsometer enables quantitative, label-free, rapid, high-throughput and low-cost analysis of biological interactions, hence the use of imaging ellipsometer coupled with microfluidic device can be utilized for uncovering the molecular mechanism of membrane fusion (Cai *et al,* 2014). Recently Nguyen *et al*., (2016) used the optogenetic oligomerization of Rab GTPases to study the intracellular membrane trafficking.

Mass spectrometry and imaging analysis of nanoparticle containing vesicles provide a mechanistic insight into cellular trafficking. Mass spectrometry technique coupled with the imaging (Hofmann *et al*., 2014; Dong *et al*., 2016, Li *et al*., 2016) and use of the fluorescence correlation spectroscopy to study the protein trafficking dynamics also holds promise. Ratiometric fluorescence imaging assay in intracellular vesicle trafficking (Samalova *et al*., 2006), semi-synthetic protein probes can also be used to understand the mechanism of targeting of Rab GTPases (Wu et al., 2010). Recently Zhang *et al*., (2017) combined the Raman imaging and multivariate analysis in order to visualize lignin, cellulose and hemicellulose in the plant cell walls. It would be interesting to see if the same technique can be used to study wild type and transgenic fruits at different developmental stages.

The use of systems and synthetic biology in the intracellular membrane trafficking of the proteins involved in the formation, development or dissolution of cell wall of the model plants as well as target crops may eventually help in understanding the highly complex process of cell wall development and intra-cellular membrane trafficking in plants. Osorio *et al*., (2011) used systems biology to study the tomato fruit development by combining the transcript, protein and metabolite analysis of *nor, rin* and *Nr* mutants. A metabolomics approach has been variously applied to discern the dynamics of metabolic processes in fruits (Carrari *et al*., 2006; Klie *et al*., 2013). Modelling of the

mechanisms of GTP hydrolysis and anchoring also provides valuable data (Carvalho *et al*., 2015) and has also been used in studying the metabolic programming in tomato fruit development (Colombié *et al*, 2015; Li and Thomas 2016). Image analysis combined with machine learning was also employed in tomato research (Yamamoto *et al*, 2017).

Vesicle proteomics can offer valuable insights into the cargo that vesicles carry during cytokinesis. Functional studies to uncover what materials RAB proteins traffic to the cell plate, the timing of delivery, and which RABs reside on common vesicles will help to fully understand their roles in cell plate formation (Worden *et al*., 2012, Darakaki *et al*., 2012). The role of Rab GTPases in modifying the cell wall of different plants for biofuel production has also been reported (Lunn *et al*., 2013; Taylor-Teeples *et al*., 2014 Voshol *et al*., 2015; Latarullo *et al*., 2016)

Rab GTPases have also been reported to act in cell divison, cell plate formation, abiotic stress tolerance and in disease resistance. Rab GTPases involved in post-Golgi trafficking to the PM are thought to be involved in plant defense (Speth *et al*., 2009), regulation of the autophagy and in hypersensitive response (Kwon *et al*., 2013). Certain pathogens also exploit or subvert RAB11-mediated trafficking e.g. *Salmonella enterica* subsp. *enterica* serovar Typhimurium uses the exocyst to recruit new membrane material to sites of bacterial attachment (to invadosomes) to increase the invasion efficiency (Nicholas *et al*., 2010)**,** whereas some viruses, such as hantavirus, hijack recycling endosomes for their release. Involvement of Rab GTPase in trafficking for growth and pathogenicity of *Fusarium graminearum* was reported by Zheng *et al*., (2015). The late blight resistance assay in the transgenic potato lines over expressing 4 StRab significantly reduced the lesion area on inoculation with *Phytophthora infestans*, suggesting role of StRab in resistance against *P. infestans* (Gao *et al*., 2012). Similarly small GTPase MoSec4 was reported to be involved in the vegetative development and pathogenicity in *Magnaporthe oryzae* (Zheng *et al*., 2016), Inada *et al*., (2016) reported that Rab GTPases were important in membrane trafficking in plants and obligate biotrophic pathogens. As the preliminary observation indicated the less susceptibility of the transgenic fruits to botrytis it would be interesting to conduct experiment based either on

artificial inoculation of the spores to test the hypothesis and to understand the underlying mechanism.

The use of protein-protein interaction (PPI) and co-expression network analysis could also find other interacting partners and effectors of small GTPases (Sun *et al*., 2017). The preliminary results of the PPI of individual Rab GTPases through STRING database (results not included in the thesis considering the volume of the thesis, but will be published later) revealed the interaction of Rab11a with LRK and PiP kinases which opens a new front for further analysis of individual classes of Rab GTPases to find their interaction and co-expression.

Analysis for Exocyst (Zárský *et al*., 2013), SNARE (Sanderfoot *et al*., 2007) and prenylated acceptors of Rab GTPases (PRA) proteins was not included in the present thesis considering the volume. PRA genes have been reported in Arabidopsis (Lee *et al*., 2011, Alvim Kamei *et al*., 2008 Rho *et al*., 2009) and in rice (Lee *et al*., 2017). It would be interesting to see if highly fruit specific promoters can be utilized for generating and screening the Rab GTPase mutants. Recently the other group of Rab GTPases was also reported in other organisms e.g. RabX and RABL (Chandra and Datta 2016, Eliáš *et al*., 2016; Zhang *et al*., 2016 RabL), it would be interesting to see if there are any RabX or RabL in tomato as well.

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TIGR DFCI-Tomato Gene Index database

TIGR DFCI TGI: http://compbio.dfci.harvard.edu/tgi/

http://compbio.dfci.harvard.edu/tgi/cgi-bin/tgi/gimain.pl?gudb=tomato

Sol genomics network,SGN : http://solgenomics.net/

TED: http://ted.bti.cornell.edu/

NCBI: http://www.ncbi.nlm.nih.gov/

TAIR Database: https://www.arabidopsis.org/

Softwares/Tools

ClustalX : http://www.clustal.org/clustal2/

Mega: http://www.megasoftware.net/

Inter-proscan : http://www.ebi.ac.uk/interpro/

Panther: http://www.pantherdb.org/

Swiss Modeller: http://swissmodel.expasy.org/?pid=smh01

Inter-proscan http://www.ebi.ac.uk/Tools/pfa/iprscan/

Swiss modeller and viewer (http://swissmodel.expasy.org/?pid=smh01).

ClustalX2 was used.ClustalX2 (http://www.clustal.org/ and http://www.clustal.org/clustal2/

Appendix

Note from the TIGR DFCI Tomato Gene Index-TGI

The computational Biology and Functional Genomics Laboratory, Dana-Farber Cancer Institute (DFCI) The Gene Indices (TGI)

The funding for this project ended in 2010 and while we continued to maintain the website, both the hardware and software supporting it have begun to fail. Effective July 15, 2014, we have suspended operation of the TGI as we lack the funds to maintain it properly. http://compbio.dfci.harvard.edu/tgi/

The software tools that enabled the TGI are still available through the DFCI Gene Indices Software Tools (ftp://occams.dfci.harvard.edu/pub/bio/tgi/software/) page.

The entire data sets, including the raw, trimmed sequences, Tensus Consensus (TC) sequences, and other data are available by FTP at

ftp://occams.dfci.harvard.edu/pub/bio/tgi/data/.
ftp://occams.dfci.harvard.edu/pub/bio/tgi/software/
ftp://occams.dfci.harvard.edu/pub/bio/tgi/data/
ftp://occams.dfci.harvard.edu/pub/bio/tgi/data/Solanum_lycopersicum/

Details of ITAG Release on Solgenomics website for tomato genome database
Source: (https://solgenomics.net/oldhighlights.pl)

Updated ITAG version 3.2 available!! (ITAG3.2)
We have updated ITAG annotation for tomato to fix some fused gene models. The updated ITAG version 3.2 is available on JBrowse, Blast and FTP. **[June 15, 2017]**

Updated ITAG version 3.1 available!! (ITAG3.1)
We have updated ITAG to fix some fused gene models. The updated ITAG version 3.1 is available on JBrowse, Blast and FTP. **[March 3, 2017].**

Improved Tomato genome assembly (SL3.0) and annotation (ITAG3.0)
We are pleased to announce the release of version SL3.0 of the tomato genome and version ITAG3.0 of the annotation. **[February 1, 2017]**

Tomato genome assembly release 2.5 available (ITAG2.4)
The tomato genome assembly 2.5 with annotation ITAG2.4, is available. The scaffolds have been resized and rearranged using FISH data from Shearer et al. (2014). **[June 21, 2014]**

Clustal parameter for multiple sequence alignments

Gap open penalty: 10

Gap extension penality: 0.2

Protein weight matrix BLOSUM62

Gap separation distance:4

Analysis

Analysis ---------- Phylogeny Reconstruction

Statistical Method ---------- Maximum Likelihood

Phylogeny Test

Test of Phylogeny ---------- Bootstrap method

No. of Bootstrap Replications --- 1000

Substitution Model

Substitutions Type ---------- Amino acid

Model/Method ---------- Jones-Taylor-Thornton (JTT) model

Rates and Patterns

Rates among Sites ---------- Uniform rates

Data Subset to Use

Gaps/Missing Data Treatment ----- Complete deletion

Tree Inference Options

ML Heuristic Method ---------- Nearest-Neighbor-Interchange (NNI)

Initial Tree for ML ---------- Make initial tree automatically (Default - NJ/BioNJ)

Branch Swap Filter ---------- Very Strong System Resource Usage

LeRab11 NCBI Report including domain information

Rab11 GTPase [Solanum lycopersicum]

NCBI Reference Sequence: NP_001233798.1

LOCUS NP_001233798 218 aa linear PLN 30-NOV-2014

DEFINITION Rab11 GTPase [Solanum lycopersicum].

ACCESSION NP_001233798

VERSION NP_001233798.1 GI:350537783

DBSOURCEREFSEQ: accession NM_001246869.1

KEYWORDS RefSeq.

SOURCE *Solanum lycopersicum* (*Lycopersicon esculentum*)

ORGANISM Solanum lycopersicum Eukaryota; Viridiplantae; Streptophyta; Embryophyta; tracheophyta; Spermatophyta; Magnoliophyta; eudicotyledons; Gunneridae; Pentapetalae; asterids; lamiids; Solanales; Solanaceae; Solanoideae; Solaneae; Solanum; Lycopersicon.

REFERENCE 1 (residues 1 to 218)

AUTHORS Rehman,R.U., Stigliano,E., Lycett,G.W., Sticher,L., Sbano,F., Faraco,M., Dalessandro,G. and Di Sansebastiano,G.P.

TITLE Tomato Rab11a characterization evidenced a difference between SYP121-dependent and SYP122-dependent exocytosis

JOURNAL Plant Cell Physiol. 49 (5), 751-766 (2008)

PUBMED 18385165

REMARK GeneRIF: The results suggest that Rab11 regulates anterograde transport from the TGN to the plasma membrane and strongly implicate SYP122, rather than SYP121.

REFERENCE 2 (residues 1 to 218)

AUTHORS Lu,C., Zainal,Z., Tucker,G.A. and Lycett,G.W.

TITLE Developmental abnormalities and reduced fruit softening in tomato plants expressing an antisense Rab11 GTPase gene

JOURNAL Plant Cell 13 (8), 1819-1833 (2001)

PUBMED 11487695

COMMENT PROVISIONAL REFSEQ: This record has not yet been subject to final NCBI review. The reference sequence was derived from AJ245570.1.

##Evidence-Data-START## Transcript exon combination :: AJ245570.1 [ECO:0000332]
RNAseq introns :: single sample supports all introns SAMN01166123, SAMN01166124 [ECO:0000348]
##Evidence-Data-END##

FEATURES Location/Qualifiers

source 1..218
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/cultivar="Ailsa Craig"
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/map="7"

Protein 1..218
/product="Rab11 GTPase"
/function="putative role in secretion of cell wall modifying enzymes"
/calculated_mol_wt=24059

Region 1..218
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Region 11..175
/region_name="Rab11_like"
/note="Rab GTPase family 11 (Rab11)-like includes Rab11a, Rab11b, and Rab25; cd01868"
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Site 11..15
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Site 20..27
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Site order(22..28,39,42,45,71,126..127,129,156..158)
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Site order(28..39,41..44)
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Site order(38,43..51)

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binding]"
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Site 45
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Site order(46,48..50,65,67,74..75,78,82,84..87,174)
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Site order(46..47,49,67..68,75,77,79..81)
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At vs LeRab11a or LeRabA1a Blast Results in TIGR Database

```
Query AT1G06400.1___Symbols__ARA2__ATRABA1A__ATRAB11E__ARA-2___ARA-2__GTP_bin
    ding___chr1_1951089-1952686_REVERSE_(217 letters)

Database:  tomato 52,502 sequences; 38,095,518 total letters.
Searching....10....20....30....40....50....60....70....80....90....100% done

                                                                    Smallest
                                                                      Sum
                                                   Reading  High  Probability
Sequences producing High-scoring Segment Pairs:      Frame Score  P(N)      N

tomato|TC224574 UniRef100_Q9SCB1 Cluster: Rab11 GTPase; n... +3    867  5.0e-87    1
tomato|TC233734 homologue to UniRef100_A7QDG9 Cluster: Ch... +3    813  3.1e-81    1
tomato|TC223336 homologue to UniRef100_A7QPP6 Cluster: Ch... +2    811  5.2e-81    1
tomato|TC238379 similar to UniRef100_Q08149 Cluster: GTP-... +1    789  1.3e-78    1
tomato|TC220390 homologue to UniRef100_Q40521 Cluster: Ra... +3    788  1.9e-78    1


>tomato|TC224574 UniRef100_Q9SCB1 Cluster: Rab11 GTPase; n=1; Solanum
            lycopersicum|Rep: Rab11 GTPase - Solanum lycopersicum (Tomato)
            (Lycopersicon esculentum), complete
        Length = 1233

 Score = 867 (310.3 bits), Expect = 5.0e-87, P = 5.0e-87
 Identities = 169/219 (77%), Positives = 187/219 (85%), Frame = +3

Query:     1 MAGYRADEEYDYLFKLVLIGDSGVGKSNLLSRFTKNEFNLESKSTIGVEFAXXXXXXXXX 60
             MAGYR D+EYDYLFKLVLIGDSGVGKSNLLSRFTKNEFNLESKSTIGVEFA
Sbjct:   135 MAGYRGDDEYDYLFKLVLIGDSGVGKSNLLSRFTKNEFNLESKSTIGVEFATKSLNIDGK 314

Query:    61 XXXAQIWDTAGQERYRAITSAYYRGAVGALLIYDVTRHATFENAARWLRELRGHTDPNIV 120
                AQIWDTAGQERYRAITSAYYRGAVGALL+YDVTRH TFEN  RWL+ELR HTDPNIV
Sbjct:   315 VIKAQIWDTAGQERYRAITSAYYRGAVGALLVYDVTRHVTFENVNRWLKELRDHTDPNIV 494

Query:   121 VMLIGNKCDLRHLVAVKTEEAKAFAERESLYFMETSALDATNVENAFTEVLTQIHKIVSK 180
             VML+GNK DLRHLVAV TEEAK+ AERE+LYFMETSAL+ATNVENAFTE LTQI++IVSK
Sbjct:   495 VMLLGNKSDLRHLVAVSTEEAKSLAEREALYFMETSALEATNVENAFTEALTQIYRIVSK 674

Query:   181 RSVDGGGESAD--LPGKGETINVKEDGSVLKRMGCCSN* 217
             ++V+ G E A    P KG+TIN+K++GS  K+ GCCS+*
Sbjct:   675 KAVEAGDEGATSTAPPKGQTININKDEGSAWKKFGCCSS* 791

Nucleotide sequence of TC224574

>TC224574 TC993 TC4412 TC7897 TC13611 TC20746 TC33432 TC43699 TC55405 TC64544 TC77060
TC94791 TC102236 TC118058 TC155959 TC176217 TC199565

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Amino Acid Sequence of TC224574:
ORF 135... 790 Frame +3 ORF Length:(218 aa)  5' end: 135 3' end: 790

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CDS: Coding Sequence of TC224574
DNA:(656 bp)
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Tentative Annotation:

UniRef100_Q9SCB1 **Cluster: Rab11 GTPase; n=1; Solanum lycopersicum|Rep: Rab11 GTPase - Solanum lycopersicum (Tomato) (Lycopersicon esculentum),** *complete*

Similarity search results:

Description	Hit coverage	% ID	% Sim	Seq Left	Seq Right	Hit Left	Hit Right
UniRef100_Q9SCB1 Cluster: Rab11 GTPase; n=1; Solanum lycopersicum|Rep: Rab11 GTPase - Solanum lycopersicum (Tomato)	*complete*	99.54	99.54	135	788	1	218
homologue to UniRef100_Q01111 Cluster: Ras-related protein YPT3; n=1; Nicotiana plumbaginifolia|Rep: Ras-related protein YPT3 -	*complete*	94.95	97.71	135	788	1	218
similar to UniRef100_A5BUD1 Cluster: Putative uncharacterized protein; n=1; Vitis vinifera|Rep: Putative uncharacterized	*complete*	85.25	93.55	135	785	1	217
similar to UniRef100_Q40194 Cluster: Ras-related protein Rab11D; n=1; Lotus japonicus|Rep: Ras-related protein Rab11D - Lotus	*complete*	85.71	91.71	135	785	1	217
similar to UniRef100_Q8LJR5 Cluster: Small GTP-binding protein; n=1; Glycine max|Rep: Small GTP-binding protein - Glycine max	*complete*	85.25	90.78	135	785	1	217

Expression Report for TC224574

Total ESTs found in TC224574: 16

Cat#	Library	# of ESTs	% of library total
T1045	tomato ovary	4 <>	0.04
#NRA	Solanum lycopersicum cv Micro-Tom root	3 <>	0.01
#K3J	Solanum lycopersicum cv. Micro-Tom leaf	3 <>	0.01
T1207	tomato callus tissue	2 <>	0.01
#K3K	Solanum lycopersicum cv. Micro-Tom fruit	1 <>	0.01
T1437	tomato germinating seed	1 <>	0.03
T1391	tomato red ripe fruit	1 <>	0.03
T1481	tomato root, plant at pre-anthesis	1 <>	0.03

GO annotation for TC224574

GO id	GO term	EC#	Type	GOpep link	%sim	%cov
GO:0003924	GTPase activity	EC 3.6.5.4	M	FB|FBgn0015790	89	81
GO:0005525	GTP binding	-	M	TAIR|gene:2171789	84	76
GO:0003677	DNA binding	-	M	GR_protein|Q941S7	89	77
GO:0016023	cytoplasmic membrane-bound vesicle	-	C	MGI|MGI:99425	90	80
GO:0030140	trans-Golgi network transport vesicle	-	C	FB|FBgn0015790	89	81
GO:0005768	endosome	-	C	FB|FBgn0015790	89	81
GO:0005811	lipid particle	-	C	FB|FBgn0015790	89	81
GO:0009536	plastid	-	C	GR_protein|A2XZG2	88	80
GO:0005886	plasma membrane	-	C	TAIR|gene:2171789	84	76
GO:0005575	cellular_component	-	C	WB|WBGene00004274	89	84
GO:0005813	centrosome	-	C	FB|FBgn0015790	89	81
GO:0005802	trans-Golgi network	-	C	TAIR|gene:3353193	93	86
GO:0043195	terminal button	-	C	FB|FBgn0015790	89	81
GO:0022416	bristle development	-	B	FB|FBgn0015790	89	81
GO:0000003	reproduction	-	B	WB|WBGene00004274	89	84
GO:0016360	sensory organ precursor cell fate determination	-	B	FB|FBgn0015790	89	81
GO:0015031	protein transport	-	B	GR_protein|Q8RZ83	88	79
GO:0050807	regulation of synapse organization and biogenesis	-	B	FB|FBgn0015790	89	81
GO:0007349	cellularization	-	B	FB|FBgn0015790	89	81
GO:0000910	cytokinesis	-	B	WB|WBGene00004274	89	84
GO:0006886	intracellular protein transport	-	B	GR_protein|Q8RZ83	88	79
GO:0042052	rhabdomere development	-	B	FB|FBgn0015790	89	81
GO:0008150	biological_process	-	B	WB|WBGene00004274	89	84
GO:0048477	oogenesis	-	B	FB|FBgn0015790	89	81
GO:0006355	regulation of transcription, DNA-dependent	-	B	GR_protein|Q941S7	89	77
GO:0019730	antimicrobial humoral response	-	B	FB|FBgn0015790	89	81
GO:0009792	embryonic development ending in birth or egg hatching	-	B	WB|WBGene00004274	89	84
GO:0000160	two-component signal transduction system (phosphorelay)	-	B	GR_protein|Q941S7	89	77
GO:0007264	small GTPase mediated signal transduction	-	B	GR_protein|Q941S7	89	77
GO:0006897	endocytosis	-	B	FB|FBgn0015790	89	81
GO:0008103	oocyte microtubule cytoskeleton polarization	-	B	FB|FBgn0015790	89	81
GO:0031532	actin cytoskeleton reorganization	-	B	FB|FBgn0015790	89	81
GO:0048749	compound eye development	-	B	FB|FBgn0015790	89	81
GO:0007317	regulation of pole plasm oskar mRNA localization	-	B	FB|FBgn0015790	89	81
GO:0040007	growth	-	B	WB|WBGene00004274	89	84
GO:0002119	nematode larval development	-	B	WB|WBGene00004274	89	84

Hierarchical clustering of individual groups of tomato Rab GTPase obtained from TED database to generate heat maps is as under.

Rab11/RabA

Type of Rab	New TC	% in fruit	Probe ID
Rab11/RabA	TC204406	71.43	1-1-6.4.11.19(3') 1-1-7.1.11.19(5')
Rab11/RabA	TC207615	66.67	1-1-3.3.5.9(5')
	TC201753	60.00	1-1-1.1.9.17(3')
Rab11/RabA	**TC200149**	57.14	1-1-7.1.8.5(3')
Rab11/RabA	**TC195610**	55.56	1-1-7.1.8.5(3') 1-1-7.1.8.5(5')
Rab11/RabA	TC193549	54.55	1-1-3.3.5.9(3')
Rab11/RabA	**TC207551**	40.00	1-1-8.4.11.20(5')
Rab11/RabA	TC212051	33.33	1-1-1.1.9.17(3')
Rab11/RabA	**TC208438**	31.25	1-1-3.3.5.9(5')
Rab11/RabA	**TC198194**	27.27	1-1-7.4.20.14(5')
	TC202127	26.67	1-1-1.4.6.16(3')
Rab11/RabA	**TC199565**	21.43	1-1-3.3.15.15(3') 1-1-3.3.15.15(5')
Rab11/RabA	TC205638	20.00	1-1-7.1.11.19(5')
Rab11/RabA	TC202193	20.00	1-1-7.1.11.19(5')
Rab11/RabA	**TC202193**	20.00	1-1-7.1.11.19(5')
Rab11/RabA	**TC208692**	18.18	1-1-8.4.19.10(5') 1-1-8.4.19.10(3')
Rab11/RabA	**TC207267**	16.67	1-1-3.3.15.15(5')
Rab11/RabA	TC192047	16.00	1-1-3.3.5.9(5')

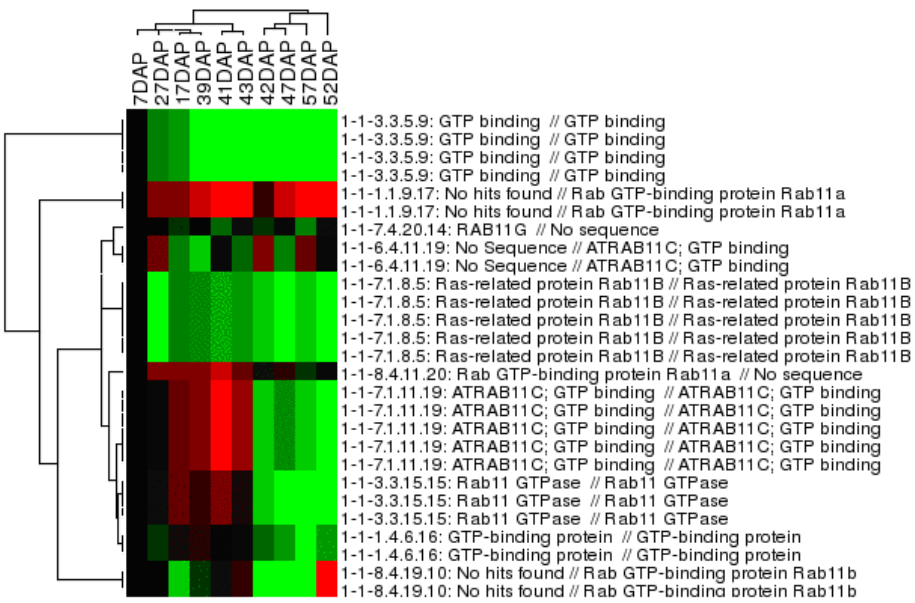
TC ids from TIGR Database, Probe Ids from TED database

The highlighted TC199565 is LeRab11a which was used in the present investigation for comparison in the wild type and antisense transgenic tomato lines.

The discrepancy between the TIGR and TED database was observed, many of the TIGR TC Ids had the same probe IDs in TED.

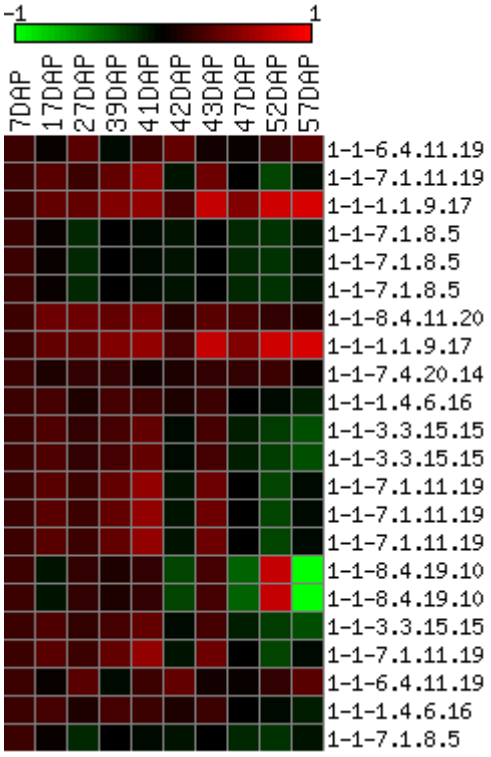
TC208692 with probe d 1-1.8.4.19.10 is strongly expressed at 52 DAP.

The Probe ID 1-1.7.1.11.19 is expressed highly at early fruit development stages 7-42 DAP and its expression declines later at red ripe stages of fruit ripening



Hierarchical clustering of all Genes corresponding to the Probe ids of LeRab11 GTPase from TED.

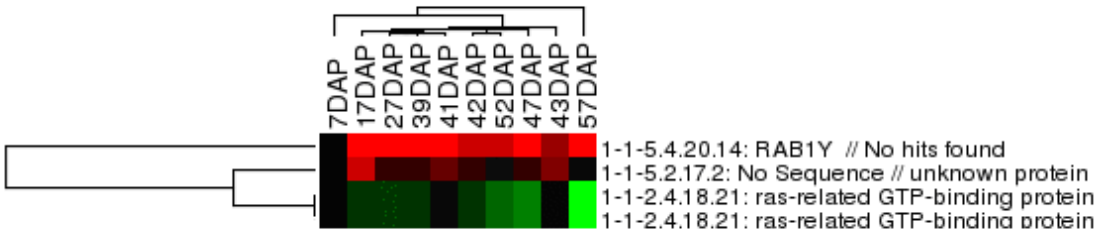
The figure shows the fruit developmental stages from 7 to 57 DAP (days after pollination). The probe id 1-1-3.3.15.15 which corresponds to LeRab11/LeRabA1a is highly expressed in early fruit development from 7 to 43 DAP immature green stage to mature green stage and declines after ripening stage from47 to 57 DAP.



K-Means clustering of all Genes corresponding to the Probe ids of LeRab 11 GTPase from TED. The expression of majority of Rab11 GTPases is higher in the early fruit development stages as shown in the heat map generated by using the K-means clustering and the expression declines after red ripe stage i.e. at52 and 57 DAP.

RabD/Rab1

Old TC	New TC	%	Probe ID from TED
TC172978	66.67	TC199235	1-1-7.2.14.18
TC178895	66.67	TC206013	1-1-5.4.20.14
TC175258	53.85	TC197849	1-1-2.4.18.21
TC191282	50.00	TC217052	1-1-5.2.17.2
TC172978	40.91	TC196657	1-1-2.4.18.21
TC175806	31.25	TC203008	1-1-5.2.17.2 (3')
TC170107	25.00	TC194670	1-1-7.2.14.18
TC178895	20.00	TC198096	1-1-5.4.20.14
TC170420	14.81	TC193154	1-1-7.2.14.18
	11.11	TC193170	1-1-7.2.14.18
TC170014	9.09	TC193212	1-1-7.2.14.18(5')

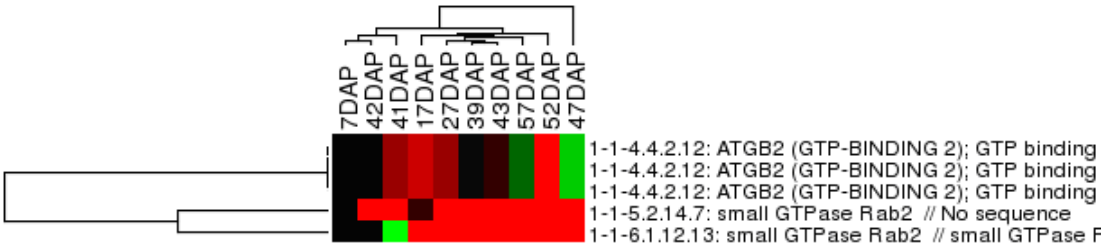


Hierarchical clustering of all Genes corresponding to the Probe ids of Le Rab 1 GTPase from TED.

Rab1 with TC206013 with probe id 1-1-5.4.20.14 is expressed highly throughout the developmental stages. Similarly the expression is higher for TC217052 with probe id 1-1-5.2.17.2 which was also shown to be expressed higher in red ripe stages in TIGR-EST database.

Rab 2/B

Old TC	New TC	%	Probe ID from TED
TC170492	TC197445	60.00	1-1-5.2.14.7(5')
TC187092	TC201999	50.00	1-1-4.4.2.12(3')
TC178011	TC194358	38.89	1-1-4.4.2.12(5')
TC187092	TC194358	38.89	1-1-4.4.2.12(5')
TC175074	TC198255	20.00	1-1-6.1.12.13(3')

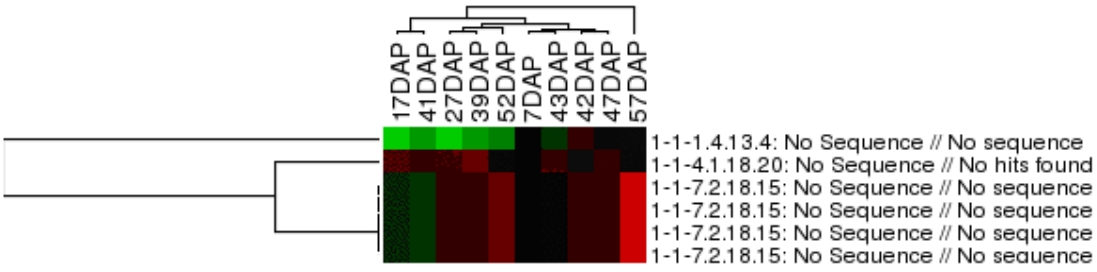


Hierarchical clustering of all Genes corresponding to the Probe ids of LeRab2 GTPase from TED.

In case of Rab2 the TC194358 and TC198255 with corresponding Probe id 1-1-5.2.14.7 and 1-1-6.12.13 both are up regulated from 17 to 57 DAP, whereas 1-1-4.2.12 is expressed higher at 17, 27, 41 and 52 DAP and down regulated at 47 and 57 DAP.

Rab5/F

Accession no.	New TC	%	Probe ID from TED
TC176115	TC199568	38.10	1-1-4.1.18.20(3')
TC182542	TC209025	33.33	1-1-1.4.13.4(3')
TC177152	TC202099	22.22	1-1-7.2.18.15(3') 1-1-7.2.18.15(5')
TC172108	TC193961	20.00	No hits found
TC177152	TC204357	0.00	1-1-7.2.18.15(3') 1-1-7.2.18.15(5')

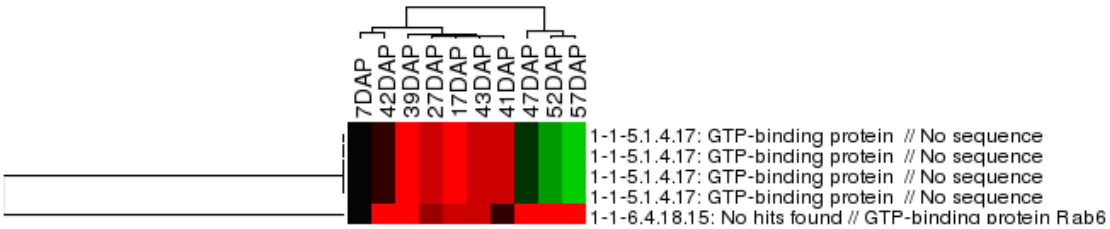


Hierarchical clustering of all Genes corresponding to the Probe ids of LeRab5 GTPase from TED.

Rab5 with TC202099 and TC204357 with the probe ids 1-1-7.2.18.15 both are up-regulated at 57 DAP whereas TC209025 with probe id 1-1-4.13.4 was down regulated from17 to 52 DAP.

Rab6/H

Accession no.	New TC	%	Probe ID from TED
TC187308	TC213216	75.00	1-1-5.1.4.17(5')
TC183750	TC210040	66.67	1-1-5.1.4.17(5')
TC177750	TC193790	50.00	1-1-5.1.4.17(5')
TC187358	TC213255	50.00	1-1-5.1.4.17(5')
TC184604	TC210789	31.03	1-1-6.4.18.15(3')

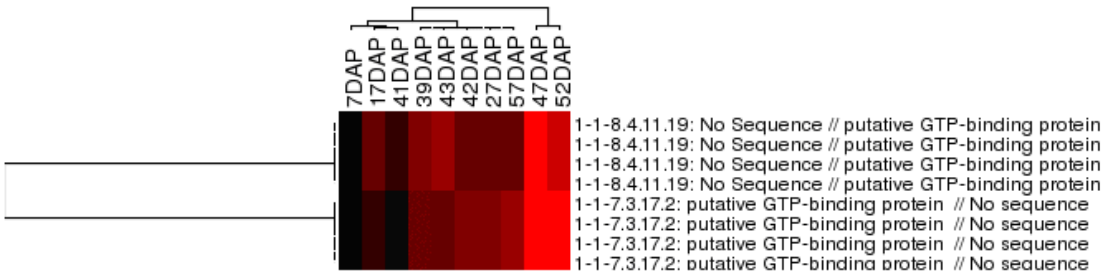


Hierarchical clustering of all Genes corresponding to the Probe ids of LeRab6 GTPase from TED.

TC213216, TC210040 with probe ids 1-1-5.1.4.17 was up-regulated at 17, 27, 39, 41, 43 DAP and was down regulated at 47, 52 and 57 DAP i.e at late red ripe stages. TC210789 with probe id 1-1-6.4.18.15 was shown to be up-regulated from 17 to 57 DAP.

Rab 7/G

Accession no.	New TC	%	New TC	Probe ID from TED	Type of Rab
TC172410	TC209067	45.61	TC209067	1-1-8.4.11.19(3')	Rab7/RabG
TC181072	TC209067	45.61	TC209067	1-1-8.4.11.19(3')	Rab7/RabG
TC181072	TC194527	45.45	TC194527	1-1-8.4.11.19(3')	Rab7/RabG
TC174722	TC199544	33.33	TC199544	1-1-7.3.17.2(5')	Rab7/RabG
TC177740	TC197104	20.00	TC197104	1-1-7.3.17.2(5')	Rab7/RabG
TC171871	TC197334	7.14	TC197334	1-1-7.3.17.2(5')	Rab7/RabG
TC177559	TC204750	0.00	TC204750	1-1-8.4.11.19(3')	Rab7/RabG
TC177740	TC206081	0.00	TC206081	1-1-7.3.17.2(5')	Rab7/RabG

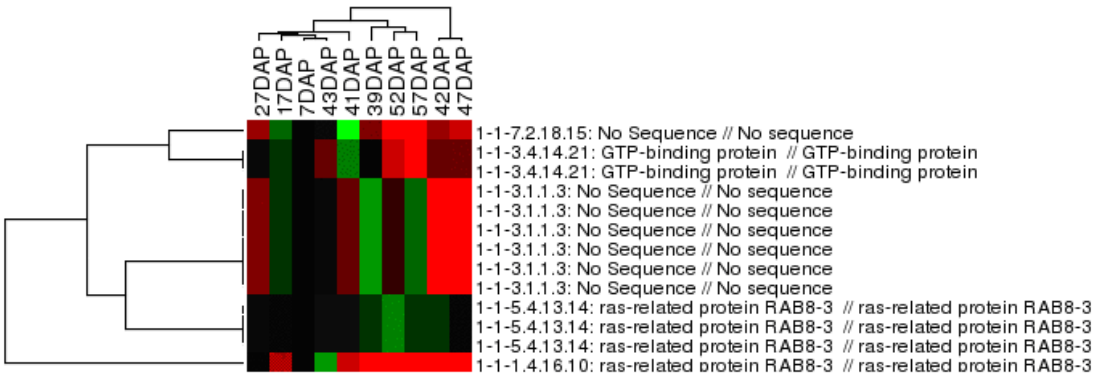


Hierarchical clustering of all genes corresponding to the Probe ids of LeRab7 GTPase from TED.

The expression of 1-1-7.3.17.2 and 1-1-8.4.11.19 was shown to be higher at 47 and 52 DAP.

Rab 8/E

TC170347	TC193111	30.77	TC193111	1-1-7.2.18.15(3')	**Rab8/RabE**
TC171031	TC192325	29.73	TC192325	1-1-3.4.14.21(5')	**Rab8/RabE**
TC174020	**TC194772**	29.41	**TC194772**	1-1-5.4.13.14(5')	**Rab8/RabE**
TC170684	TC193590	14.29	TC193590	1-1-3.1.1.3(5')	**Rab8/RabE**
TC173103	TC193590	14.29	TC193590	1-1-3.1.1.3(5')	**Rab8/RabE**
TC188589	TC193590	14.29	TC193590	1-1-3.1.1.3(5')	**Rab8/RabE**
TC171031	TC191969	8.33	TC191969	1-1-3.4.14.21(5')	**Rab8/RabE**
TC170684	TC199454	0.00	TC199454	1-1-3.1.1.3(3')	**Rab8/RabE**
TC173103	TC199454	0.00	TC199454	1-1-3.1.1.3(3')	**Rab8/RabE**
TC175489	**NA**	NA	**NA**	**NA**	**Rab8/RabE**
TC186042	**TC212065**	0.00	**TC212065**	1-1-1.4.16.10(3') 1-1-5.4.13.14(5') 1-1-5.4.13.14(3')	**Rab8/RabE**
TC190150	**TC207687**	0.00	**TC207687**	1-1-3.1.1.3(5')	**Rab8/RabE**



Hierarchical clustering of all genes corresponding to the Probe ids of Le Rab 8 GTPase from TED.

TC199454 wth probe id 1-1-3.1.1.3 was up-regulated at 42 and 47 DAP (MG stage) and was down regulated at red ripe stage at 57 DAP.

Comparison of the 12th and 13th Release of TIGR TC for Tomato Rab GTPase

RAB	Release12 Jul 2008	New TC Release 13 April 2010	CDS and ORF length	Hit coverage	% ID	% Sim	Seq Left	Seq Right	Hit Left	Hit Right
RabD/ Rab1	TC193212	TC218008	ORF 160 .. 770 Frame +1 5' end: 160 3' end: 770 ORF: (203 aa)	*complete*	100	100	160	768	1	203
	TC194670	TC219445	ORF 8 .. 795 Frame +2 5' end: 8 3' end: 795 ORF:(262 aa)	*complete*	98.52	98.52	185	793	1	203
	TC193170	TC217961	ORF 151 .. 761 Frame +1 5' end: 151 3' end: 761 ORF:(203 aa)	*complete*	100.00	100.00	151	759	1	203
	TC193154	TC217946	ORF 70 .. 677 Frame +1 5' end: 70 3' end: 677 ORF:(202 aa)	*complete*	100.00	100.00	70	675	1	202
	TC196657	TC230012	ORF 138 .. 307 Frame +3 5' end: 138 3' end: 307 ORF:(56 aa)	*partial (69%)*	100.00	100.00	2	421	64	203
		TC218685	ORF 133 .. 713 Frame +1 5' end: 133 3' end: 713 ORF:(193 aa)	*partial (94%)*	99.47	100.00	133	702	1	190
		TC222748	ORF 168 .. 748 Frame +3 5' end: 168 3' end: 748 ORF:(193 aa)	*partial (94%)*	97.37	97.89	168	737	1	190
		TC217784	ORF 92 .. 702 Frame +2 5' end: 92 3' end: 702 ORF:(203 aa)	*complete*	99.51	100.00	92	700	1	203
	TC199235	TC224211	ORF 81 .. 416 Frame framefinder 5' end: 81 3' end: 416 ORF:(111 aa)	*partial (58%)*	100.00	100.00	73	426	86	203
	TC197849	TC222748	ORF 168 .. 748 Frame +3 5' end: 168 3' end: 748 ORF:(193 aa)	*partial (94%)*	97.37	97.89	168	737	1	190
	TC203008	TC228595	ORF 477 .. 1087 Frame +3 5' end: 477 3' end: 1087 ORF:(203 aa)	*complete*	89.16	94.09	477	1085	1	203
	TC217052	TC245198	ORF 391 .. 1005 Frame ESTScan (+) 5' end: 391 3' end: 1005	*partial (77%)*	94.90	96.82	391	1006	1	157

			ORF:(204 aa)							
RabC/ Rab18	TC198096	TC223016	ORF 254 .. 882 Frame +2 5' end: 254 3' end: 882 ORF:(209 aa)	*partial (98%)*	93.20	98.54	263	880	6	211
	TC206013	TC232260	ORF 202 .. 614 Frame +1 5' end: 202 3' end: 614 ORF:(137 aa)	*partial (64%)*	90.30	96.27	211	612	6	139
	TC210194	TC235984	ORF 178 .. 661 Frame +1 5' end: 178 3' end: 661 ORF:(160 aa)	*partial (71%)*	90.60	94.63	214	660	9	157
RabB/ Rab2	TC197445	TC239055	ORF 305 .. 939 Frame +2 5' end: 305 3' end: 939 ORF:(211 aa)	*complete*	100.00	100.00	305	934	1	210
	TC198255	TC223185	ORF 92 .. 726 Frame +2 5' end: 92 3' end: 726 ORF:(211 aa)	*complete*	99.52	100.00	92	721	1	210
	TC194358	TC219185	ORF 165 .. 808 Frame +3 5' end: 165 3' end: 808 ORF:(214 aa)	*complete*	92.75	95.65	165	793	1	210
	TC201999	TC242604	ORF 836 .. 202 Frame -2 5' end: 836 3' end: 202 ORF:(211 aa)	*complete*	93.75	98.08	837	208	1	208
RabF/ Rab5	TC193961	TC218706	ORF 159 .. 760 Frame +3 5' end: 159 3' end: 760 ORF:(200 aa)	*complete*	96.50	98.50	159	758	1	200
	TC204357	TC227481	ORF 155 .. 756 Frame +2 5' end: 155 3' end: 756 ORF:(200 aa)	*complete*	91.96	96.98	155	751	1	199
	TC202099	TC234866	ORF 79 .. 608 Frame +1 5' end: 79 3' end: 608 ORF:(176 aa)	*partial (84%)*	94.61	96.41	79	579	1	167
	TC209025	TC235767	ORF 165 .. 712 Frame +3 5' end: 165 3' end: 712 ORF:(182 aa)	*partial (94%)*	90.91	94.12	150	710	14	200
		TC224486	ORF 110 .. 711 Frame +2 5' end: 110 3' end: 711 ORF:(200 aa)	*complete*	90.50	93.50	110	709	1	200
	TC199568	TC224570	ORF 180 .. 781 Frame +3 5' end: 180 3' end: 781 ORF:(200 aa)	*complete*	91.88	93.40	180	770	1	197
RabH/ Rab6	TC193790	TC218546	ORF 123 .. 745 Frame +3 5' end: 123 3' end: 745 ORF:(207 aa)	*complete*	91.30	96.14	123	743	1	207

	TC210040	TC229699	ORF 215 .. 1234 Frame +2 5' end: 215 3' end: 1234 ORF:(339 aa)	*partial (95%)*	93.88	97.45	215	803	1	196
	TC210789	TC237903	ORF 183 .. 808 Frame +3 5' end: 183 3' end: 808 ORF:(208 aa)	*complete*	98.08	99.04	183	806	1	208
	TC213216	TC240704	ORF 69 .. 691 Frame +3 5' end: 69 3' end: 691 ORF:(207 aa)	*complete*	91.79	97.10	69	689	1	207
	TC213255	TC240746	ORF 164 .. 447 Frame +2 5' end: 164 3' end: 447 ORF:(94 aa)	*partial (70%)*	91.67	95.14	164	818	1	146
RabG/ Rab7	TC197334	TC222197	ORF 141 .. 757 Frame +3 5' end: 141 3' end: 757 ORF:(205 aa)	*complete*	98.54	99.51	141	755	1	205
	TC209067	TC217468	ORF 104 .. 723 Frame +2 5' end: 104 3' end: 723 ORF:(206 aa)	*complete*	100.00	100.00	104	721	1	206
	TC199544	TC224535	ORF 261 .. 727 Frame +3 5' end: 261 3' end: 727 ORF:(155 aa)	*partial (78%)*	93.75	96.88	93	718	1	160
	TC204750	TC230711	ORF 90 .. 220 Frame +3 5' end: 90 3' end: 220 ORF:(43 aa)	*partial (52%)*	98.13	100.00	2	322	99	205
	TC206081	TC220556	ORF 161 .. 774 Frame +2 5' end: 161 3' end: 774 ORF:(204 aa)	*complete*	95.59	97.06	161	772	1	204
	TC197104	TC220556	Same as TC206081							
	TC194527	TC217468	Same as TC209067							
RabE/ Rab8	TC193111	TC217915	ORF 84 .. 733 Frame +3 5' end: 84 3' end: 733 ORF:(216 aa)	*complete*	97.69	98.61	84	731	1	216
	TC193590	TC218364	ORF 156 .. 805 Frame +3 5' end: 156 3' end: 805 ORF:(216 aa)	*complete*	99.54	99.54	156	803	1	216
	TC199454	TC224448	ORF 179 .. 639 Frame +2 5' end: 179 3' end: 639 ORF:(153 aa)	*partial (71%)*	100.00	100.00	179	637	1	153
	TC192325	TC223370	ORF 386 .. 771 Frame +2 5' end: 386 3' end: 771 ORF:(128 aa)	*complete*	95.81	96.74	124	769	2	216
		TC217391	ORF 247 .. 896 Frame +1 5' end: 247 3' end: 896 ORF:(216 aa)	*complete*	99.54	100.00	247	894	1	216

	TC191969	TC224023	ORF 134 .. 783 Frame +2 5' end: 134 3' end: 783 ORF:(216 aa)	*complete*	99.54	100.00	134	781	1	216
	TC194772	TC219550	ORF 170 .. 834 Frame +2 5' end: 170 3' end: 834 ORF:(221 aa)	*complete*	94.91	97.69	170	816	1	216
	TC212065	TC239367	ORF 97 .. 482 Frame +1 5' end: 97 3' end: 482 ORF:(128 aa)	*partial (74%)*	94.97	96.86	4	480	58	216
	TC207687	TC234213	ORF 263 .. 801 Frame +2 5' end: 263 3' end: 801 ORF:(179 aa)	*complete*	92.09	95.81	154	799	1	216
RabA/Rab 11	TC202193	TC227595	ORF 115 .. 742 Frame +1 5' end: 115 3' end: 742 ORF:(208 aa)	*partial (95%)*	94.09	95.57	115	738	1	206
	TC195734	TC220527	ORF 54 .. 703 Frame +3 5' end: 54 3' end: 703 ORF:(216 aa)	*complete*	93.84	95.73	54	701	1	214
	TC193549	TC223336	ORF 86 .. 738 Frame +2 5' end: 86 3' end: 738 ORF:(217 aa)	*complete*	93.98	98.61	86	733	1	216
	TC207615	TC234136	ORF 209 .. 758 Frame +2 5' end: 209 3' end: 758 ORF:(182 aa)	*partial (84%)*	91.21	95.60	209	754	1	182
	TC200149	TC223058	ORF 211 .. 887 Frame +1 5' end: 211 3' end: 887 ORF:(225 aa)	*partial (84%)*	83.15	94.02	223	783	4	189
	TC200286	TC225354	ORF 3 .. 295 Frame +3 5' end: 3 3' end: 295 ORF:(97 aa)	*partial (44%)*	88.66	93.81	3	293	126	222
	TC192047	TC225814	ORF 74 .. 741 Frame +2 5' end: 74 3' end: 741 ORF:(222 aa)	*complete*	93.24	96.85	74	739	1	222
	TC199565	TC224574	ORF 135 .. 790 Frame +3 5' end: 135 3' end: 790 ORF:(218 aa)	*complete*	99.54	99.54	135	788	1	218
	TC195610	TC220390	ORF 93 .. 745 Frame +3 5' end: 93 3' end: 745 ORF:(217 aa)	*complete*	97.22	99.07	93	740	1	216
	TC198194	TC223129	ORF 76 .. 728 Frame +1 5' end: 76 3' end: 728 ORF:(217 aa)	*complete*	94.74	99.04	100	726	1	209

RabA/ Rab11	TC207026	TC233465	ORF 57 .. 521 Frame +3 5' end: 57 3' end: 521 ORF:(154 aa)	*partial (72%)*	95.48	99.35	57	521	1	155
	TC207267	TC233734	ORF 171 .. 823 Frame +3 5' end: 171 3' end: 823 ORF:(217 aa)	*partial (98%)*	90.14	96.24	171	821	1	218
	TC205638	TC231813	ORF 206 .. 677 Frame +2 5' end: 206 3' end: 677 ORF:(156 aa)	*partial (73%)*	94.90	99.36	206	676	1	157
	TC204406	TC230320	ORF 121 .. 767 Frame +1 5' end: 121 3' end: 767 ORF:(215 aa)	*complete*	89.30	95.35	121	765	1	215
	TC207551	TC234079	ORF 633 .. 1196 Frame +3 5' end: 633 3' end: 1196 ORF:(187 aa)	*partial (84%)*	92.02	96.81	633	1196	1	188
	TC208438	TC235051	ORF 177 .. 850 Frame +3 5' end: 177 3' end: 850 ORF:(224 aa)	*complete*	96.35	98.63	192	848	4	222
	TC208692	TC235365	ORF 1339 .. 661 Frame framefinder (-) 5' end: 1339 3' end: 661 ORF:(225 aa)	*partial (96%)*	86.85	93.43	806	128	1	223
	TC212051	TC239355	ORF 94 .. 635 Frame +1 5' end: 94 3' end: 635 ORF:(180 aa)	*partial (91%)*	83.08	92.82	6	591	3	197
	TC201753	TC227076	ORF 154 .. 704 Frame +1 5' end: 154 3' end: 704 ORF:(183 aa)	*partial (85%)*	84.07	93.96	157	702	3	184
	TC211111	TC224630	ORF 228 .. 517 Frame +3 5' end: 228 3' end: 517 ORF:(96 aa)	*partial (79%)*	92.40	97.66	3	515	47	217
	TC202127	TC227506	ORF 118 .. 770 Frame +1 5' end: 118 3' end: 770 ORF:(217 aa)	*complete*	90.78	97.70	118	768	1	217
	TC211213	TC238379	ORF 145 .. 794 Frame +1 5' end: 145 3' end: 794 ORF:(216 aa)	*complete*	88.32	95.79	145	789	1	216

Table shows the Comparison of the 12th and 13th Release of TIGR TC for Tomato Rab GTPase. The column shows the Rab GTPase class, old TC, new TC, CDS and ORF details, hit coverage in percentage, percent ID, percent similarity and details of hit on left and right side of sequence.

Expression values of Rab11/RabA GTPase group with Solyc IDs from SGN database at IMG, BR and red ripe fruits.

Solyc Ids of Rab11/A GTPase	Immature green fruit:BR	Mature green fruit:BR	Breaker fruit:BR	Fully ripe fruit:BR	Min	Max	Average	Standard Deviation	Max Fold Change
Solyc01g086850.2.1	1.356	2.679	2.503	1.991	1.356	2.679	2.132	0.515	1.976
Solyc01g096220.2.1	3.934	4.345	4.888	1.382	1.382	4.888	3.637	1.345	3.537
Solyc01g103380.2.1	2.939	3.237	2.947	2.317	2.317	3.237	2.86	0.336	1.397
Solyc02g072180.2.1	2.457	0.752	0.259	-1.521	-1.521	2.457	0.487	1.417	-
Solyc03g006270.2.1	NA	-3.051	-4.21	-2.528	-4.21	-2.528	-3.263	0.703	-
Solyc03g079900.2.1	-1.92	-3.647	-2.048	-0.812	-3.647	-0.812	-2.107	1.011	-
Solyc03g118820.2.1	0.589	2.903	4.109	2.923	0.589	4.109	2.631	1.276	6.976
Solyc05g052900.2.1	NA	NA	NA	NA	-	-	-	-	-
Solyc05g054150.2.1	3.504	2.905	1.977	-1.81	-1.81	3.504	1.644	2.067	-
Solyc06g005810.2.1	1.899	1.46	1.734	0.94	0.94	1.899	1.508	0.364	2.02
Solyc06g076450.2.1	2.174	2.452	2.689	3.064	2.174	3.064	2.595	0.327	1.409
Solyc07g053480.2.1	4.159	2.205	1.246	-0.96	-0.96	4.159	1.663	1.842	-
Solyc07g055290.2.1	2.375	2.793	2.51	1.949	1.949	2.793	2.407	0.304	1.433
Solyc09g056340.2.1	3.103	3.736	4.764	2.633	2.633	4.764	3.559	0.798	1.809
Solyc09g097900.2.1	1.65	1.236	1.304	-0.632	-0.632	1.65	0.89	0.892	-
Solyc09g098170.2.1	3.836	3.167	3.42	2.841	2.841	3.836	3.316	0.364	1.35
Solyc10g079030.1.1	NA	NA	NA	NA					

Rab GTPase in SGN according to Sol ITAG Release 2.4

Blast Result tomato 10G045550

Sequences producing significant alignments:	Score (bits)	E value	
Solyc10g045550.1.1 Ras-related protein Rab-25 IPR015595 Rab11-related	441	1.00E-158	Rab11
Solyc01g103380.2.1 Ras-related protein Rab-25 IPR015595 Rab11-related	397	1.00E-141	Rab11
Solyc06g005810.2.1 Ras-related protein Rab-25 IPR015595 Rab11-related	318	1.00E-110	Rab11
Solyc06g076450.2.1 Ras-related protein Rab-25 IPR015595 Rab11-related	318	1.00E-110	Rab11
Solyc03g079900.2.1 Ras-related protein Rab-25 IPR015595 Rab11-related	315	1.00E-109	Rab11
Solyc07g053480.2.1 Ras-related protein Rab-25 IPR015595 Rab11-related	314	1.00E-108	Rab11
Solyc12g010790.1.1 Ras-related protein Rab-25 IPR015595 Rab11-related	309	1.00E-106	Rab11
Solyc05g052900.2.1 Ras-related protein Rab-25 IPR015595 Rab11-related	308	1.00E-106	Rab11
Solyc03g006270.2.1 Ras-related protein Rab-25 IPR015595 Rab11-related	303	1.00E-104	Rab11
Solyc12g014080.1.1 Ras-related protein Rab-25 IPR015595 Rab11-related	303	1.00E-104	Rab11
Solyc07g055290.2.1 Ras-related protein Rab-25 IPR015595 Rab11-related	303	1.00E-104	Rab11
Solyc02g072180.2.1 Ras-related protein Rab-25 IPR015595 Rab11-related	301	1.00E-103	Rab11
Solyc10g008840.2.1 Ras-related protein Rab-25 IPR015595 Rab11-related	300	1.00E-103	Rab11
Solyc11g010100.1.1 Ras-related protein Rab-25 IPR015595 Rab11-related	281	1.00E-95	Rab11
Solyc04g012180.2.1 Ras-related protein Rab-25 IPR015595 Rab11-related	276	2.00E-93	Rab11
Solyc01g086850.2.1 Ras-related protein Rab-25 IPR015595 Rab11-related	270	3.00E-91	Rab11
Solyc10g079030.1.1 Ras-related protein Rab-25 IPR015595 Rab11-related	263	3.00E-88	Rab11
Solyc03g064020.2.1 Ras-related protein Rab-25 IPR015595 Rab11-related	259	6.00E-87	Rab11
Solyc01g096220.2.1 Ras-related protein Rab-25 IPR015595 Rab11-related	249	2.00E-82	Rab11
Solyc05g054150.2.1 Ras-related protein Rab-25 IPR015595 Rab11-related	248	3.00E-82	Rab11
Solyc11g012460.1.1 Ras-related protein Rab-25 IPR015595 Rab11-related	245	1.00E-80	Rab11
Solyc09g056340.2.1 Ras-related protein Rab-25 IPR015595 Rab11-related	243	2.00E-80	Rab11
Solyc09g098170.2.1 Ras-related protein Rab-25 IPR015595 Rab11-related	237	4.00E-78	Rab11
Solyc03g118820.2.1 Ras-related protein Rab-25 IPR015595 Rab11-related	236	1.00E-77	Rab11
Solyc09g097900.2.1 Ras-related protein Rab-25 IPR015595 Rab11-related	231	9.00E-76	Rab11
Solyc10g050030.1.1 Ras-related protein Rab-25 IPR015595 Rab11-related	106	4.00E-29	Rab11
Solyc08g078070.2.1 Ras-related protein Rab-1A IPR003579 Ras small GTPase, Ra...	202	8.00E-65	Rab1
Solyc05g052070.2.1 Ras-related protein Rab-1A IPR002078 RNA polymerase	201	2.00E-64	Rab1
Solyc05g053940.2.1 Ras-related protein Rab-1A IPR003579 Ras small GTPase, Ra...	197	1.00E-62	Rab1
Solyc01g090170.2.1 Ras-related protein Rab-1A IPR003579 Ras small GTPase, Ra...	192	6.00E-61	Rab1
Solyc01g103370.2.1 Ras-related protein Rab-1A IPR003579 Ras small GTPase, Ra...	187	9.00E-59	Rab1
Solyc12g011130.1.1 Ras-related protein Rab-2-A IPR003579 Ras small GTPase, R...	192	1.00E-60	Rab2
Solyc10g007700.2.1 Ras-related protein Rab-2-A IPR003579 Ras small GTPase, R...	190	5.00E-60	Rab2
Solyc02g093530.2.1 Ras-related protein Rab-2-A IPR003579 Ras small GTPase, R...	190	6.00E-60	Rab2
Solyc07g056150.2.1 Ras-related protein Rab-2-A IPR003579 Ras small GTPase, R...	171	2.00E-52	Rab2
Solyc06g060210.1.1 Ras-related protein Rab-2-A IPR003579 Ras small GTPase, R...	168	1.00E-51	Rab2
Solyc11g073050.1.1 Ras-related protein Rab-8A IPR003579 Ras small GTPase, Ra...	181	2.00E-56	Rab8
Solyc04g011360.2.1 Ras-related protein Rab-8A IPR003579 Ras small GTPase, Ra...	178	2.00E-55	Rab8
Solyc10g086350.1.1 Ras-related protein Rab-8A IPR003579 Ras small GTPase, Ra...	178	4.00E-55	Rab8
Solyc09g010370.2.1 Ras-related protein Rab-8A IPR003579 Ras small GTPase, Ra...	176	2.00E-54	Rab8
Solyc06g005350.2.1 Ras-related protein Rab-8A IPR003579 Ras small GTPase, Ra...	155	5.00E-47	Rab8
Solyc02g069370.2.1 Ras-related protein Rab-5C IPR015599 Rab5-related	167	5.00E-51	Rab5
Solyc11g008430.1.1 Ras-related protein Rab-5C IPR015599 Rab5-related	158	2.00E-47	Rab5
Solyc02g081380.2.1 Ras-related protein Rab-5C IPR015599 Rab5-related	155	1.00E-46	Rab5
Solyc02g036450.2.1 Ras-related protein Rab-5C IPR015599 Rab5-related	154	4.00E-46	Rab5
Solyc05g045660.2.1 Ras-related protein Rab-5C IPR003579 Ras small GTPase, Ra...	6.39E+01	1.00E-12	Rab5
Solyc10g006380.1.1 Ras-related protein Rab-21 IPR003579 Ras small GTPase, Ra...	6.08E+01	3.00E-11	Rab5
Solyc04g064510.2.1 Ras-related protein Rab-18 IPR015598 Rab18	146	5.00E-43	Rab18

Solyc09g008460.2.1 Ras-related protein Rab-18 IPR015598 Rab18	143	9.00E-42	Rab18
Solyc11g008020.1.1 Ras-related protein Rab-18 IPR015598 Rab18	142	3.00E-41	Rab18
Solyc10g086310.1.1 Ras-related protein Rab-18 IPR015598 Rab18	140	2.00E-40	Rab18
Solyc03g078570.2.1 Ras-related protein Rab-6A IPR015600 Rab6-related	142	2.00E-41	Rab6
Solyc01g088560.2.1 Ras-related protein Rab-6A IPR015600 Rab6-related	141	6.00E-41	Rab6
Solyc05g051570.2.1 Ras-related protein Rab-6A IPR015600 Rab6-related	140	1.00E-40	Rab6
Solyc01g109520.2.1 Ras-related protein Rab-7 IPR002078 RNA polymerase sigma .	134	2.00E-38	Rab7
Solyc03g120750.2.1 Ras-related protein Rab-7 IPR003579 Ras small GTPase, Rab...	131	2.00E-37	Rab7
Solyc04g072060.2.1 Ras-related protein Rab-7a IPR003579 Ras small GTPase, Ra...	131	3.00E-37	Rab7
Solyc04g051680.2.1 Ras-related protein Rab-7 IPR003579 Ras small GTPase, Rab...	122	1.00E-33	Rab7
Solyc01g104700.2.1 GTP-binding nuclear protein Ran-A1 IPR018210 GTP-binding	97.1	3.00E-24	Ran
Solyc01g104680.2.1 GTP-binding nuclear protein Ran-A1 IPR018210 GTP-binding .	97.1	3.00E-24	Ran
Solyc05g023800.2.1 GTP-binding nuclear protein Ran-A1 IPR002041 Ran GTPase	97.1	3.00E-24	Ran
Solyc01g111560.2.1 Ras-like GTP-binding protein RHO IPR003578 Ras small GTPase...	90.1	8.00E-22	Rop
Solyc01g088780.2.1 Rho-related GTP-binding protein RhoC IPR003578 Ras small	89.7	1.00E-21	Rop
Solyc02g062020.2.1 Ras-like GTP-binding protein RHO IPR003578 Ras small GTPase...	87.8	6.00E-21	Rop
Solyc02g077400.2.1 Rac-like GTP binding protein IPR003578 Ras small GTPase, ...	8.63E+01	2.00E-20	Rop
Solyc02g083580.2.1 Ras-like GTP-binding protein RHO IPR003578 Ras small GTPase...	8.59E+01	3.00E-20	Rop
Solyc03g033420.2.1 Rho-related GTP-binding protein RhoC IPR003578 Ras small ...	8.36E+01	2.00E-19	Rop
Solyc03g114070.2.1 Rac-like GTP-binding protein 4 IPR003578 Ras small GTPase...	8.13E+01	1.00E-18	Rop
Solyc07g020800.2.1 Rac-like GTP-binding protein 4 IPR003578 Ras small GTPase...	8.09E+01	2.00E-18	Rop
Solyc12g007210.1.1 Ras-like GTP-binding protein RHO IPR003578 Ras small GTPase...	7.66E+01	6.00E-17	Rop

List of Thesis Supplemental files in DVD

DFCI Gene Indices Information Page.doc
DFCI GI.doc
LeGI Release Notes.doc
List of Rabs TIGR DFCI TC.doc
New Le Rab NT and AA Sequences ORF from the TIGR DFCI TGI .doc
New TIGR vs SGN IDs.doc
NT, CDS & ORF Seq for New Le Rab TC from TIGR + annotation.doc
old Class of Rab TIGR TC and SGN ID.doc
Solanum lycopersicum Gene Ontology Assignments.doc
TBLASTN 2 tigr tc vs sgn unigene ids.doc
Tomato Rab GTPase Amino Acid Sequence.doc
Le ARF GTPase
Le ARL GTPase
Le Rab GAP
Le RAB GDI
Le RAB GEF
Le RAB GTPase Fasta Files
Le RAN GTPase
Le ROP GTPase
Le SAR GTPase
Rab GTPase Expression Profiles
Rab GTPase Primer Validation and Blast Results
Rab GTPase Protein Structure Prediction
Rab GTPase Sequence Analysis
Rab GTPase TC Reports TIGR DFCI TGI Release 11
Results At Vs Sl Rab GTPase BLAST TIGR-Release 13 Jan 2011
Results Small GTPase BLAST TIGR 2011
Results Small GTPase BLAST TIGR July 2010
SGN Solyc Blast from TC
TIGR At VS Le Blast SGN Unigene-Mar-2011
TIGR DFCI TGI March 2014
TIGR vs SGN UNIgene
Tomato RAB,RAN,ROP,SAR, ARF, ARL, GAP, GEF, GDI Blast TIGR TC-SGN 2011
\KS Thesis SPL Files Jan 2018\Le ARF GTPase
ARF GTPase.xls
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Expression Profiles of New Probe Ids from TED.doc
Expression Report for Le Rab TIGR DFCI TGI.doc
Expr_Profiles_of_Probe_IDs_from_TED_Oct_2008.doc
Heat map by K clustering.doc
K-means Clustering of Le Rab.doc
Le Rab Primers for QRT PCR.doc
LeRab11 TIGR Expr.doc
Microarray Expression Database 24 April 2008.doc
New Expression of Le Rab in Fruits from the new TIGR TC.doc
New Expression of LeRab in Fruits from the new TIGR TC.doc
New TC Expression Report for Le Rab.doc
Rab 11 Tomato Array Probe Annotation.doc
Relative expression data retrieval.doc
Relative Expression of probe ids 100 percent match.doc
TED search result for Rab and GTPase.doc
TIGR New TC expression in fruits.xls
Tomato Probe Expression profiles Oct 2008.doc
Tomato Gene Expression Data Development stages
Tomato tissue specific genes
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Copy of Tomato ripening induced genes.xls
Differentially expressed genes
normalized_data.xls
RAW DATA WT Set 1
Tomato pathogenesis-related genes.xls
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Flower specific genes.xls	Root specific genes.xls
Fruit specific genes modified 9-Jan-2008.xls	Seed specific genes.htm
Ripening fruit specific genes.xls	Shoot specific genes.htm
Suspension culture specific genes.xls	Pollen specific genes.xls
Trichome specific genes.htm	
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Le Rab 7	
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model3.gif	
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Motif Scan Results Tomato Rab11.doc	
MUSTER Results for Tomato Rab11 GTPase.doc	
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RCSB Structure Viewers	QQQp_1.ribbons
Taxonomic distribution of proteins with RAB domain.doc	RibbonsReadMe.doc
TMpred output for Le Rab11a.doc	Ribbons_Manual.pdf
University of Illinois Software	SPDBV_4.01_PC
visualization of protein Polyview3d.pdf	Swiss model server results.doc
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\KS Thesis SPL Files Jan 2018\Rab GTPase Sequence Analysis
1 GO prt bndng.doc
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Rab 11 Tomato Array Probe Annotation.doc
Rab and AA seq.doc
Rab GTPase Amino Acid Composition Analysis.xls
Rab GTPase Sequence Analysis Files txt-fasta-dnd-phb files
Rab GTPase Sequence Analysis-Bioedit
Rab GTPase Sequence Analysis-Clustal X2
Rab GTPase Sequence Analysis-ClustalW2
Rab GTPase Sequence Analysis-MEGA
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TGN transport Rab SNAREs n Sars.doc

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\KS Thesis SPL Files Jan 2018\Results Small GTPase BLAST TIGR 2011\Le ARL	
Le ARL GTPase seq info.doc	
New At vs Le ARL prt fasta.aln	New At vs Le ARL prt fasta.phb
New At vs Le ARL prt fasta.dnd	New At vs Le ARL prt fasta.txt
Query AT2G18390.1.doc	Query AT5G37680.1.txt
Query AT2G18390.1.txt	Query AT5G52210.1.doc
Query AT3G49860.1.doc	Query AT5G52210.1.txt
Query AT3G49860.1.txt	Query AT5G52210.2.doc
Query AT3G49870.1.doc	Query AT5G52210.2.txt
Query AT3G49870.1.txt	Query AT5G67560.1.doc
Query AT5G37680.1.doc	Query AT5G67560.1.txt
\KS Thesis SPL Files Jan 2018\Results Small GTPase BLAST TIGR 2011\LE RAB GAP	
At Rab GAP prt tabular.txt	
New At vs Le Rab GAP prt Fasta.aln	New At vs Le Rab GAP prt Fasta.phb
New At vs Le Rab GAP prt Fasta.dnd	New At vs Le Rab GAP prt Fasta.txt
New At vs Le Rab GAP prt Fasta.ph	New Le Rab GAP seq info.doc
Query AT1G04830.1.doc	Query AT3G55020.1.txt
Query AT1G04830.1.txt	Query AT3G57590.1.doc
Query AT2G20440.1.doc	Query AT3G57590.1.txt
Query AT2G20440.1.txt	Query AT4G13730.1.doc
Query AT2G30710.1.doc	Query AT4G13730.1.txt
Query AT2G30710.1.txt	Query AT4G27100.1.doc
Query AT2G37290.1.doc	Query AT4G27100.1.txt
Query AT2G37290.1.txt	Query AT4G28550.1.doc
Query AT2G39280.1.doc	Query AT4G28550.1.txt
Query AT2G39280.1.txt	Query AT5G15930.1.doc
Query AT2G43490.1.doc	Query AT5G15930.1.txt
Query AT2G43490.1.txt	Query AT5G24390.1.doc
Query AT3G02460.1.doc	Query AT5G24390.1.txt
Query AT3G02460.1.txt	Query AT5G41940.1.doc
Query AT3G07890.1.doc	Query AT5G41940.1.txt
Query AT3G07890.1.txt	Query AT5G53570.1.doc
Query AT3G49350.1.doc	Query AT5G53570.1.txt
Query AT3G49350.1.txt	Query AT5G54780.1.doc
Query AT3G55020.1.doc	Query AT5G54780.1.txt
\KS Thesis SPL Files Jan 2018\Results Small GTPase BLAST TIGR 2011\Le RAB GDI	
New At vs Le Rab GDI prt fasta.aln	New At vs Le Rab GDI prt fasta.phb
New At vs Le Rab GDI prt fasta.dnd	New At vs Le Rab GDI prt fasta.txt
New Le Rab GDI Seq Info.doc.docx	
Query AT3G06540.1.txt	Query AT3G06540.1.doc
Query AT2G44100.1.txt	Query AT3G59920.1.doc
Query AT2G44100.2.doc	Query AT3G59920.1.txt
Query AT2G44100.2.txt	Query AT5G09550.1.doc
Query AT2G44100.doc	Query AT5G09550.1.txt

\KS Thesis SPL Files Jan 2018\Results Small GTPase BLAST TIGR 2011\Le RAB GEF	
New At vs Le Rab GEF TGI prt Fasta.aln	New At vs Le Rab GEF TGI prt Fasta.phb
New At vs Le Rab GEF TGI prt Fasta.dnd	New At vs Le Rab GEF TGI prt Fasta.txt
New Le Rab GEF TGI Results.doc	
Query AT3G19770.1.txt	Query AT3G19770.doc
Query AT3G19770.2.doc	Query AT5G09320.1.doc
Query AT3G19770.2.txt	Query AT5G09320.1.txt
\KS Thesis SPL Files Jan 2018\Results Small GTPase BLAST TIGR 2011\Le RAB GTPase	
At Rab GTPase Prt Fasta.txt	sl vs at prt fasta oct.dnd
At vs Sl SGN ALGN domains.bmp	sl vs at prt fasta oct.ph
New Sl rab GTPase Prt Fasta TIGR - Copy.fasta	sl vs at prt fasta.phb
New Sl rab GTPase Prt Fasta TIGR.txt	sl vs at prt fasta.txt
OrthoMCL_result_tomITAGa2.40v2.2preV2.3_athT9_rap2_vit_potITAG.txt	slrabtreemxlklhd.mts
slrabtreemxlklhd2crcl.mts	
\KS Thesis SPL Files Jan 2018\Results Small GTPase BLAST TIGR 2011\Le RAN	
At vs Le Ran GTPase prt Fasta.aln	At vs Le Ran GTPase prt Fasta.txt
At vs Le Ran GTPase prt Fasta.dnd	At vs Le Ran GTPase prt Fasta.phb
New Le RAN GTPase seq info.doc.docx	
Query AT5G20010.1.txt	Query AT5G55080.1.doc
Query AT5G20010.doc	Query AT5G55080.1.txt
Query AT5G20020.1.doc	Query AT5G55190.1.doc
Query AT5G20020.1.txt	Query AT5G55190.1.txt
\KS Thesis SPL Files Jan 2018\Results Small GTPase BLAST TIGR 2011\Le ROP	
New At vs Le ROP prt Fasta.aln	New At vs Le ROP prt Fasta.txt
New At vs Le ROP prt Fasta.dnd	New Le ROP seq Info TGI.doc
New At vs Le ROP prt Fasta.phb	
Query AT1G20090.1.txt	Query AT3G51300.1.txt
Query AT1G20090.doc	Query AT4G28950.1.doc
Query AT1G75840.1.doc	Query AT4G28950.1.txt
Query AT1G75840.1.txt	Query AT4G35020.1.doc
Query AT2G17800.1.doc	Query AT4G35020.1.txt
Query AT2G17800.1.txt	Query AT4G35950.1.doc
Query AT2G17800.2.doc	Query AT4G35950.1.txt
Query AT2G17800.2.txt	Query AT5G45970.1.txt
Query AT2G44690.1.doc	Query AT5G45970.doc
Query AT2G44690.1.txt	Query AT5G62880.1.doc
Query AT3G48040.1.doc	Query AT5G62880.1.txt
Query AT3G48040.1.txt	Query AT3G51300.1.doc
\KS Thesis SPL Files Jan 2018\Results Small GTPase BLAST TIGR 2011\Le SAR	
At vs Le SAR GTPase prt Fasta.aln	At vs Le SAR GTPase prt Fasta.phb
At vs Le SAR GTPase prt Fasta.dnd	At vs Le SAR GTPase prt Fasta.txt
lesarjalviewalign.png	
New Le SAR GTPase TIGR DFCI TGI Seq results.doc	
Query AT1G56330.1.txt	Query AT1G09180.doc
Query AT1G56330.doc	Query AT4G02080.1.txt
Query AT1G09180.1.txt	Query AT4G02080.doc

SGN Query AT1G09180.1.doc	TED Query AT1G09180.1.doc
SGN Query AT1G56330.1.doc	TED Query AT1G56330.1.doc
SGN Query= AT4G02080.1.doc	TED Query AT4G02080.1.doc
\KS Thesis SPL Files Jan 2018\Results Small GTPase BLAST TIGR July 2010	
Le ARF	Le RAN
Le ARL	Le ROP
LE RAB GAP	Le SAR
Le RAB GDI	Le RAB GEF
\KS Thesis SPL Files Jan 2018\Results Small GTPase BLAST TIGR July 2010\Le ARF	
Query AT1G02430.1.txt	Query AT3G03120.1.doc
Query AT1G02430.doc	Query AT3G03120.1.txt
Query AT1G02440.1.doc	Query AT3G22950.1.doc
Query AT1G02440.1.txt	Query AT3G22950.1.txt
Query AT1G10630.1.doc	Query AT3G62290.1.doc
Query AT1G10630.1.txt	Query AT3G62290.1.txt
Query AT1G23490.1.doc	Query AT5G14670.1.doc
Query AT1G23490.1.txt	Query AT5G14670.1.txt
Query AT1G70490.1.doc	Query AT5G17060.1.doc
Query AT1G70490.1.txt	Query AT5G17060.1.txt
Query AT2G15310.1.doc	Query AT2G15310.1.txt
\KS Thesis SPL Files Jan 2018\Results Small GTPase BLAST TIGR July 2010\Le ARL	
Query AT2G18390.1.doc	Query AT5G37680.1.txt
Query AT2G18390.1.txt	Query AT5G52210.1.doc
Query AT3G49860.1.doc	Query AT5G52210.1.txt
Query AT3G49860.1.txt	Query AT5G52210.2.doc
Query AT3G49870.1.doc	Query AT5G52210.2.txt
Query AT3G49870.1.txt	Query AT5G67560.1.doc
Query AT5G37680.1.doc	Query AT5G67560.1.txt
\KS Thesis SPL Files Jan 2018\Results Small GTPase BLAST TIGR July 2010\LE RAB GAP	
Query AT1G04830.1.doc	Query AT3G55020.1.txt
Query AT1G04830.1.txt	Query AT3G57590.1.doc
Query AT2G20440.1.doc	Query AT3G57590.1.txt
Query AT2G20440.1.txt	Query AT4G13730.1.doc
Query AT2G30710.1.doc	Query AT4G13730.1.txt
Query AT2G30710.1.txt	Query AT4G27100.1.doc
Query AT2G37290.1.doc	Query AT4G27100.1.txt
Query AT2G37290.1.txt	Query AT4G28550.1.doc
Query AT2G39280.1.doc	Query AT4G28550.1.txt
Query AT2G39280.1.txt	Query AT5G15930.1.doc
Query AT2G43490.1.doc	Query AT5G15930.1.txt
Query AT2G43490.1.txt	Query AT5G24390.1.doc
Query AT3G02460.1.doc	Query AT5G24390.1.txt
Query AT3G02460.1.txt	Query AT5G41940.1.doc
Query AT3G07890.1.doc	Query AT5G41940.1.txt
Query AT3G07890.1.txt	Query AT5G53570.1.doc
Query AT3G49350.1.doc	Query AT5G53570.1.txt

Query AT3G49350.1.txt	Query AT5G54780.1.doc
Query AT3G55020.1.doc	Query AT5G54780.1.txt
\KS Thesis SPL Files Jan 2018\Results Small GTPase BLAST TIGR July 2010\Le RAB GDI	
Query AT3G06540.1.txt	Query AT3G06540.1.doc
Query AT2G44100.1.txt	Query AT3G59920.1.doc
Query AT2G44100.2.doc	Query AT3G59920.1.txt
Query AT2G44100.2.txt	Query AT5G09550.1.doc
Query AT2G44100.doc	Query AT5G09550.1.txt
\KS Thesis SPL Files Jan 2018\Results Small GTPase BLAST TIGR July 2010\Le RAB GEF	
Query AT3G19770.1.txt	Query AT3G19770.doc
Query AT3G19770.2.doc	Query AT5G09320.1.doc
Query AT3G19770.2.txt	Query AT5G09320.1.txt
\KS Thesis SPL Files Jan 2018\Results Small GTPase BLAST TIGR July 2010\Le RAN	
Query AT5G20010.1.txt	Query AT5G55080.1.doc
Query AT5G20010.doc	Query AT5G55080.1.txt
Query AT5G20020.1.doc	Query AT5G55190.1.doc
Query AT5G20020.1.txt	Query AT5G55190.1.txt
\KS Thesis SPL Files Jan 2018\Results Small GTPase BLAST TIGR July 2010\Le ROP	
Query AT1G20090.1.txt	Query AT4G28950.1.doc
Query AT1G20090.doc	Query AT4G28950.1.txt
Query AT1G75840.1.doc	Query AT4G35020.1.doc
Query AT1G75840.1.txt	Query AT4G35020.1.txt
Query AT2G17800.1.doc	Query AT4G35950.1.doc
Query AT2G17800.1.txt	Query AT4G35950.1.txt
Query AT2G17800.2.doc	Query AT5G45970.1.txt
Query AT2G17800.2.txt	Query AT5G45970.doc
Query AT2G44690.1.doc	Query AT5G62880.1.doc
Query AT2G44690.1.txt	Query AT5G62880.1.txt
Query AT3G48040.1.doc	Query AT3G51300.1.doc
Query AT3G48040.1.txt	Query AT3G51300.1.txt
\KS Thesis SPL Files Jan 2018\Results Small GTPase BLAST TIGR July 2010\Le SAR	
Query AT1G56330.1.txt	Query AT1G09180.doc
Query AT1G56330.doc	Query AT4G02080.1.txt
Query AT1G09180.1.txt	Query AT4G02080.doc
SGN Query AT1G09180.1.doc	TED Query AT1G09180.1.doc
SGN Query AT1G56330.1.doc	TED Query AT1G56330.1.doc
SGN Query= AT4G02080.1.doc	TED Query AT4G02080.1.doc
\KS Thesis SPL Files Jan 2018\SGN Solyc Blast from TC	
All Solyc Rab GTPase Prt.txt	
Rab1	Rab5
Rab11	Rab6
Rab18	Rab7
Rab2	Rab8
Solyc blast Rab1-8 combined.txt	Solyc blast Rab11 combined.txt
\KS Thesis SPL Files Jan 2018\SGN Solyc Blast from TC\Rab1	
Query TC193154.txt	Query TC193212.txt

Query TC193170.txt	Query TC194670.txt
Query TC197849.txt	Query TC196657.txt
Query TC199235 chk length.txt	Query TC203008.txt
Query TC217052 rab1.txt	
\KS Thesis SPL Files Jan 2018\SGN Solyc Blast from TC\Rab11	
Query TC192047 Rab11.txt	Query TC195610 Rab11.txt
Query TC193549 Rab11.txt	Query TC195734 Rab11.txt
Query TC199565 LeRab11a.txt	Query TC198194 Rab11.txt
Query TC200149 Rab11.txt	Query TC207267 Rab11.txt
Query TC200286 Rab11 chk length.txt	Query TC207551 Rab11.txt
Query TC201753 Rab11.txt	Query TC207615 Rab11.txt
Query TC202127 Rab11.txt	Query TC208438 Rab11.txt
Query TC202193 Rab11.txt	Query TC208692 Rab11.txt
Query TC204406 Rab11.txt	Query TC211111 Rab11.txt
Query TC205638 Rab11.txt	Query TC211213 RAb11.txt
Query TC207026 Rab11.txt	Query TC212051 Rab11.txt
Solyc Rab11 protein seq fasta with id only and seq.txt	
\KS Thesis SPL Files Jan 2018\SGN Solyc Blast from TC\Rab18	
Query TC198096 Rab18.txt	Query TC206013 Rab18.txt
\KS Thesis SPL Files Jan 2018\SGN Solyc Blast from TC\Rab2	
Query TC194358 Rab2.txt	Query TC197445 Rab2.txt
Query TC201999 Rab2 chk length.txt	Query TC198255 Rab2.txt
\KS Thesis SPL Files Jan 2018\SGN Solyc Blast from TC\Rab5	
Query TC193961 Rab5.txt	Query TC204357 Rab5.txt
Query TC199568 Rab5.txt	Query TC209025 Rab5.txt
Query TC202099 Rab5.txt	
\KS Thesis SPL Files Jan 2018\SGN Solyc Blast from TC\Rab6	
Query TC193790 Rab6.txt	Query TC210789 Rab6.txt
Query TC210040 Rab6.txt	Query TC213216 Rab6.txt
\KS Thesis SPL Files Jan 2018\SGN Solyc Blast from TC\Rab7	
Query TC194527 Rab7.txt	Query TC197334 Rab7.txt
Query TC197104 Rab7.txt	Query TC199544 Rab7.txt
Query TC209067 Rab7.txt	
\KS Thesis SPL Files Jan 2018\SGN Solyc Blast from TC\Rab8	
Query TC191969 Rab8.txt	Query TC194772 Rab8.txt
Query TC192325 Rab8.txt	Query TC199454 Rab8.txt
Query TC193111 Rab8.txt	Query TC207687 Rab8.txt
Query TC193590 Rab8.txt	Query TC212065 Rab8.txt
\KS Thesis SPL Files Jan 2018\TIGR At VS Le Blast SGN Unigene-Mar-2011	
At vs Le ARF GTPase Unigene SGN Tabular.txt	At vs Le Rab GAP Unigene SGN.txt
At vs Le ARF GTPase Unigene SGN.txt	At vs Le Rab GDI Unigene SGN.txt
At vs Le ARL GTPase Unigene SGN Tabular.txt	At vs Le Rab GEF Unigene SGN.txt
At vs Le ARL GTPase Unigene SGN.txt	At vs Le Rab Unigene SGN.txt
At vs Le Rab GAP Unigene SGN Tabular.txt	At vs Le Ran Unigene SGN.txt
At vs Le Rab GDI Unigene SGN Tabular.txt	At vs Le ROP Unigene SGN.txt

At vs Le Rab GEF Unigene SGN Tabular.txt	At vs Le SAR GTPase SGN.txt
At vs Le SAR GTPase Unigene SGN Tabular.txt	At vs Le Rab Unigene SGN Tabular.txt
At vs Le SAR GTPase Unigene SGN.txt	At vs Le Ran Unigene SGN Tabular.txt
At vs Le ROP Unigene SGN Tabular.txt	
\KS Thesis SPL Files Jan 2018\TIGR DFCI TGI March 2014	
Sl Rab1-D	Sl Rab5-F
Sl Rab11-A	Sl Rab6-H
Sl Rab18-C	Sl Rab7-G
Sl Rab2-B	Sl Rab8-E
\KS Thesis SPL Files Jan 2018\TIGR DFCI TGI March 2014\Sl Rab1-D	
At VS SlRAB1-D and 18 C prt fasta.aln	At VS SlRAB1-D prt fasta.aln
At VS SlRAB1-D and 18 C prt fasta.dnd	At VS SlRAB1-D prt fasta.dnd
At VS SlRAB1-D and 18 C prt fasta.phb	At VS SlRAB1-D prt fasta.fasta
At VS SlRAB1-D prt fasta TEST .aln	At VS SlRAB1-D prt fasta.mas
At VS SlRAB1-D prt fasta TEST .dnd	At VS SlRAB1-D prt fasta.phb
At VS SlRAB1-D prt fasta TEST .pdf	At VS SlRAB1-D prt fasta TEST .ps
At VS SlRAB1-D prt fasta TEST .phb	At VS SlRAB1-D prt fasta TEST.aln
At VS SlRAB1-D prt fasta TEST.dnd	
At VS SlRAB1-D.txt	
AT1G02130-ATRABD2a	AT4G17530-ATRABD2C
AT3G11730-ATRABD1	AT5G47200-ATRABD2B
RabD all 4 hits table.txt	
SlRAB1-D.aln	SlRAB1-D.ps
SlRAB1-D.dnd	SlRAB1-D.txt
\KS Thesis SPL Files Jan 2018\TIGR DFCI TGI March 2014\Sl Rab1-D\AT1G02130-ATRABD2a	
AT1G02130-ATRABD2A.docx	
AT1G02130-ATRABD2A.txt	
\KS Thesis SPL Files Jan 2018\TIGR DFCI TGI March 2014\Sl Rab1-D\AT3G11730-ATRABD1	
AT3G11730-ATRABD1.docx	AT3G11730-ATRABD1.txt
TC217784.txt	TC218685.txt
TC217946.txt	TC219445.txt
TC217961.txt	TC222748.txt
TC218008.txt	TC228595.txt
TC218364.txt	TC245198.txt
\KS Thesis SPL Files Jan 2018\TIGR DFCI TGI March 2014\Sl Rab1-D\AT4G17530-ATRABD2C	
AT4G17530-ATRABD2C.docx	AT4G17530-ATRABD2C.txt
\KS Thesis SPL Files Jan 2018\TIGR DFCI TGI March 2014\Sl Rab1-D\AT5G47200-ATRABD2B	
AT5G47200-ATRABD2B.docx	AT5G47200-ATRABD2B.txt
\KS Thesis SPL Files Jan 2018\TIGR DFCI TGI March 2014\Sl Rab11-A	
\KS Thesis SPL Files Jan 2018\TIGR DFCI TGI March 2014\Sl Rab18-C	
At VS Sl RAB18-C.meg	At VS Sl-RAB18-C.mas
At VS Sl-RAB18-C.fasta	At VS Sl-RAB18-C.txt
AT1G43890.1-ATRABC1	AT5G03530-ATRABC2A
AT3G09910-ATRABC2B	SLRAB18-C.txt
\KS Thesis SPL Files Jan 2018\TIGR DFCI TGI March 2014\Sl Rab18-C\AT1G43890.1-ATRABC1	
AT1G43890-ATRABC1.docx	TC232260.txt

AT1G43890-ATRABC1.txt	TC235984.txt
TC223016.txt	TC236038.txt
\KS Thesis SPL Files Jan 2018\TIGR DFCI TGI March 2014\Sl Rab18-C\AT3G09910-ATRABC2B	
AT3G09910-ATRABC2B.docx	AT3G09910-ATRABC2B.txt
\KS Thesis SPL Files Jan 2018\TIGR DFCI TGI March 2014\Sl Rab18-C\AT5G03530-ATRABC2A	
AT5G03530-ATRABC2A.docx	AT5G03530-ATRABC2A.txt
\KS Thesis SPL Files Jan 2018\TIGR DFCI TGI March 2014\Sl Rab2-B	
\KS Thesis SPL Files Jan 2018\TIGR DFCI TGI March 2014\Sl Rab5-F	
\KS Thesis SPL Files Jan 2018\TIGR DFCI TGI March 2014\Sl Rab6-H	
\KS Thesis SPL Files Jan 2018\TIGR DFCI TGI March 2014\Sl Rab7-G	
\KS Thesis SPL Files Jan 2018\TIGR DFCI TGI March 2014\Sl Rab8-E	
\KS Thesis SPL Files Jan 2018\TIGR vs SGN Unigene	
Query= TC193154.doc	Query= TC198096.doc
Query= TC193170.doc	Query= TC199235.doc
Query= TC193212.doc	Query= TC203008.doc
Query= TC194670.doc	Query= TC206013.doc
Query= TC196657.doc	Query= TC217052.doc
Query= TC197849.doc	
\KS Thesis SPL Files Jan 2018\Tomato RAB,RAN,ROP,SAR, ARF, ARL, GAP, GEF, GDI Blast TIGR TC-SGN 2011	
At vs Le ARF GTPase Unigene SGN Tabular.txt	At vs Le Rab GAP Unigene SGN.txt
At vs Le ARF GTPase Unigene SGN Tabular.xls	At vs Le Rab GDI Unigene SGN.txt
At vs Le ARF GTPase Unigene SGN.txt	At vs Le Rab GEF Unigene SGN.txt
At vs Le ARL GTPase Unigene SGN Tabular.txt	At vs Le Rab Unigene SGN.txt
At vs Le ARL GTPase Unigene SGN Tabular.xls	At vs Le Ran Unigene SGN.txt
At vs Le ARL GTPase Unigene SGN.txt	At vs Le ROP Unigene SGN.txt
At vs Le Rab GAP Unigene SGN Tabular.txt	At vs Le SAR GTPase SGN.txt
At vs Le Rab GAP Unigene SGN Tabular.xls	At vs Le Ran Unigene SGN Tabular.txt
At vs Le Rab GDI Unigene SGN Tabular.txt	At vs Le Rab Unigene SGN Tabular.txt
At vs Le Rab GDI Unigene SGN Tabular.xls	At vs Le Rab Unigene SGN Tabular.xls
At vs Le Rab GEF Unigene SGN Tabular.txt	At vs Le Ran Unigene SGN Tabular.xls
At vs Le Rab GEF Unigene SGN Tabular.xls	At vs Le ROP Unigene SGN Tabular.txt
At vs Le Rab GEF Unigene SGN Tabular.xlsx	At vs Le ROP Unigene SGN Tabular.xls
At vs Le Rab SGN PAIRWISE MULTIPLE BLAST RESULTS.txt	At vs Le SAR GTPase Unigene SGN Tabular.txt
At vs Le SAR GTPase Unigene SGN Tabular.xls	At vs Le SAR GTPase Unigene SGN.txt
SGN Solyc ITAG Sequence Analysis	
At vs Solyc ALL Rab GTPase Prt seq.aln	All Solyc Rab GTPase prt seq.aln
At vs Solyc ALL Rab GTPase Prt seq.dnd	All Solyc Rab GTPase prt seq.dnd
At vs Solyc ALL Rab GTPase Prt seq.ph	All Solyc Rab GTPase prt seq.ph
At vs Solyc ALL Rab GTPase Prt seq.phb	All Solyc Rab GTPase prt seq.phb
At vs Solyc ARL & SAR Mega Session Tree.mts	At vs Solyc ARL.jvp
At vs Solyc ARL Mega tree session.mts	At vs Solyc RabGDI Jalview session.jvp
At vs Solyc Rab GAP tree session MEGA.mts	At vs Solyc ARF Mega session tree.mts
At vs Solyc GDI Aligenment Pfam format.pfam	At vs Solyc ROP with At Names.mts
At vs Solyc RAN GTPase Jalview MSA.jvp	
SGN Solyc BLAST Results At TAIR Vs Solyc ITAG	

\KS Thesis SPL Files Jan 2018\SGN Solyc BLAST Results At TAIR Vs Solyc ITAG	
Rab1-RabD	Rab5-RabF
Rab11-RabA	Rab6-RabH
Rab18-RabC	Rab7-RabG
Rab2-RabB	Rab8-RabE
Rab GAP	Rab GEF
Rab GDI	ARF
ARL	RAN
SAR	ROP
ARF	
Query AT1G02430.1 ATARFD1B.txt	Query AT2G47170.1-ARF1A1C.txt
Query AT1G02440.1 ATARFD1A.txt	Query AT3G03120.1 ATARFB1C.txt
Query AT1G10630.1 ATARFA1F.txt	Query AT3G22950.1 ATARFC1.txt
Query AT1G23490.1 ATARFA1A.txt	Query AT3G62290.1 ATARFA1E.txt
Query AT1G70490.1 ATARFA1D.txt	Query AT5G14670.1 ATARFA1B.txt
Query AT2G15310.1 ATARFB1A.txt	Query AT5G17060.1 ATARFB1B.txt
ARL	
Query AT2G18390.1 ARL2 ATARLC1.txt	Query AT5G37680.1 ATARLA1A.txt
Query AT3G49860.1 ATARLA1B.txt	Query AT5G52210.1 ATARLB1.txt
Query AT3G49870.1 ATARLA1C.txt	Query AT5G67560.1 ATARLA1D.txt
SAR	
Query AT1G09180.1 ATSARA1A.txt	Query AT4G02080.1 ATSARA1C.txt
Query AT1G56330.1 ATSARA1B.txt	
RAN	
Query AT5G20010.1 ATRAN1.txt	Query AT5G55080.1 AtRAN4.txt
Query AT5G20020.1 RAN2.txt	Query AT5G55190.1 ATRAN3.txt
ROP	
Query AT1G20090.1 ATROP2.txt	Query AT3G51300.1 ATROP1.txt
Query AT1G75840.1 ATROP4.txt	Query AT4G28950.1 ATROP9.txt
Query AT2G17800.1 ATROP3.txt	Query AT4G35020.1 ATROP6.txt
Query AT2G44690.1 ATROP8.txt	Query AT4G35950.1 ATROP5.txt
Query AT3G48040.1 ATROP10.txt	Query AT5G45970.1 ATROP7.txt
Query AT5G62880.1 ATROP11.txt	
Rab GAP	
Query AT1G04830.1 RabGAP-TBC.txt	Query AT2G39280.1 Rab GAP.txt
Query AT2G20440.1 RabGAP-TBC.txt	Query AT2G43490.1 Rab GAP.txt
Query AT2G30710.1 RabGAP-TBC.txt	Query AT3G02460.1 AtGYPD1a.txt
Query AT2G37290.1 RabGAP-TBC.txt	Query AT3G07890.1 At GYPC1a.txt
Query AT3G57590.1 At GYPB1f CHECK F box prt anntt.txt	Query AT3G49350.1 AtGYPB2d.txt
Query AT4G13730.1 At GYPA1b.txt	Query AT3G55020.1 AtGYPc1b.txt
Query AT4G27100.1 AtGYPB1c.txt	Query AT5G24390.1 At GYPB2e.txt
Query AT4G28550.1 At GYPb1b.txt	Query AT5G41940.1 AtGYPB2b.txt
Query AT5G15930.1 AtGYPD1b.txt	Query AT5G53570.1 AtGYPB2c.txt
Query AT5G54780.1 AtGYPB1d.txt	
Rab GEF	
Query AT3G19770.1 ATVPS9A GEF rho.txt	Query AT5G09320.1 VPS9B.txt

Query AT3G19770.2 ATVPS9A Rho GEF.txt	
Rab GDI	
Query AT2G44100.1 ATGDI1,.txt	Query AT3G06540.1 At GDI.txt
Query= AT5G09550.1 AtRab GDI.txt	Query AT3G59920.1 ATGDI2.txt
Rab1-Rab-D	
Query AT1G02130.1 ATRABD2A.txt	Query AT4G17530.1 ATRABD2C.txt
Query AT3G11730.1 ATRABD1.txt	Query AT5G47200.1 ATRABD2B.txt
Rab2-RabB	
Query AT4G17160.1-ATRABB1A.txt	Query AT4G35860.1-ATRABB1B.txt
Query AT4G17170.1-ATRABB1C.txt	Query AT4G35860.2-ATRABB1B.txt
Rab5-RabF	
Query AT4G19640.1 ATRABF2B ATRAB5B.txt	Query AT3G54840.1 ATRABF1.txt
Query AT5G45130.1 ATRAB5A ATRABF2A.txt	Query AT3G54840.2 RABF1.txt
Rab6-RabH	
Query AT2G22290.1 ATRABH1D.txt	Query AT5G10260.1 AtRABH1e.txt
Query AT2G44610.1 ATRABH1B ATRAB6A.txt	Query AT5G64990.1 AtRABH1a.txt
Query AT4G39890.1 AtRABH1c.txt	
Rab7-RabG	
Query AT1G22740.1 RAB7 ATRABG3B.txt	Query AT1G52280.1 AtRABG3d.txt
Query AT1G49300.1 ATRAB7 ATRABG3E.txt	Query AT3G16100.1 ATRABG3C.txt
Query AT2G21880.1 ATRAB7A ATRABG2.txt	Query AT4G09720.1 ATRABG3A.txt
Query AT3G18820.1 ATRABG3F ATRAB7B.txt	Query AT5G39620.1 AtRABG1.txt
Rab8-RabE	
Query AT3G09900.1 ATRABE1E ATRAB8E.txt	
Query AT3G46060.1 ATRABE1C ATRAB8A.txt	
Query AT3G53610.1 AtRab8B AtRABE1a.txt	Query AT4G20360.1 NOT RAB 8.txt
Query AT5G03520.1 ATRAB8C ATRABE1D.txt	Query AT5G59840.1 At RabE1b.txt
Rab11-RabA	
Query AT1G01200-ATRABA3.txt	Query AT3G12160-ATRABA4D.txt
Query AT1G05810-ATRABA5E.txt	Query AT3G15060-AtRABA1g.txt
Query AT1G06400-ATRABA1A.txt	Query AT3G46830-ATRABA2C.txt
Query AT1G07410-ATRABA2B.txt	Query AT4G18430-AtRABA1e.txt
Query AT1G09630-ATRABA2A.txt	Query AT4G18800-ATRABA1D.txt
Query AT1G16920-ATRABA1B.txt	Query AT4G39990-ATRABA4B.txt
Query AT1G18200-AtRABA6b.txt	Query AT5G45750-AtRABA1c.txt
Query AT1G28550-AtRABA1i.txt	Query AT5G47520-AtRABA5a.txt
Query AT1G73640-AtRABA6a.txt	Query AT5G47960-ATRABA4C.txt
Query AT2G31680-AtRABA5d.txt	Query AT5G59150-ATRABA2D.txt
Query AT2G33870-ArRABA1h.txt	Query AT5G60860-AtRABA1f.txt
Query AT2G43130-ATRABA5C.txt	Query AT5G65270-AtRABA4a.txt
Query AT3G07410-AtRABA5b.txt	
Rab18-RabC	
Query AT3G09910.1 ATRAB18C ATRABC2B.txt	
Query AT1G43890.1 ATRAB18, ATRABC1.txt	
Query AT5G03530.1 ATRAB18B ATRABC2A.txt	

File Formats: (Filename) . (Extension)
* is file name
Extensions

*.txt	Text file notepad
*.aln	Alignment files
*.dnd	Clustal tree file
*.ph	Tree file
*.phb	Boostrap tree file
*.jpg or.jpeg	Image file
*.gif	Animated file
*.jvp	Java program file
*.doc or *.docx	MS Word file
*.phy	Phylip file
*.fas or .fasta	FASTA file
*.gb	Gene bank file
*.tre	Tree file
*.pdb	Protein databank file
*.mts	Mega Trees file
*.ribbons	Ribbon file
*.nwk	Newick file
*.xls	MS Excel file